Population pharmacokinetics of antibiotics to prevent group B streptococcal disease: from mother to neonate

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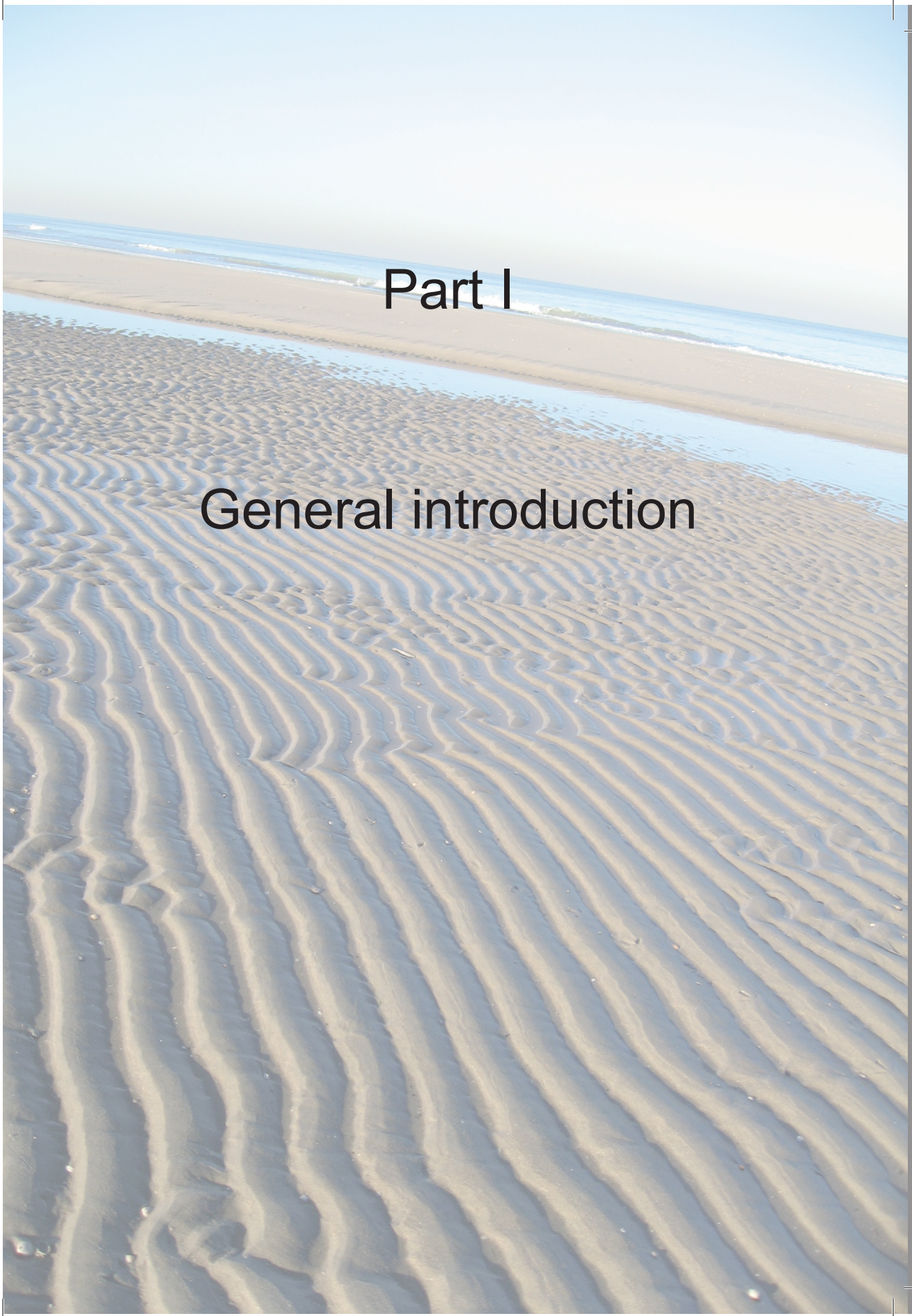
Everything is impossible until it has been done.

Dr. Tore Godal, 2005

Contents

Abbreviations

AF	Amniotic fluid
AUC	Area under the curve
BW	Body weight
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CG	Cockcroft-Gault
CI	Confidence interval
CL	Clearance
CSF	Cerebrospinal fluid
CV	Coefficient of variation
EOD	Early-onset disease
Eta	Inter-individual variation NONMEM
EUCAST	European Committee on Antimicrobial Susceptibility Testing
F	Female
f	Unbound fraction of drug
*f*T>MIC	The time the fraction not bound to proteins is above the MIC
GA	Gestational age
GBS	Group B streptococcus
GFR	Glomerular filtration rate
HPLC	High-pressure liquid chromatography
IAI	Intra-amniotic infection
IIV	Inter-individual variability
IPA	Intrapartum prophylaxis with antibiotics
LOD	Late-onset disease
M	Male
MCS	Monte Carlo Simulation
MDRD	Modification of Diet in Reneal Disease
MIC	Minimum inhibitory concentration
NONMEM	Non-Linear Mixed Effects Modeling
OFV	Objective function value
PD	Pharmacodynamics
PK	Pharmacokinetics
PPROM	Premature preterm rupture of the membranes
PROM	Preterm rupture of the membranes
PTA	Probability of target attainment
Q	Intercompartmental clearance
SD	Standard deviation
SE	Standard error
$t_{1/2}$	Half-life
theta	Parameter in NONMEM
UTI	Urinary tract infection
V	Volume of distribution
V_{ss}	Volume of distribution at steady state



Part I

General introduction

Chapter 1

Population pharmacokinetics of antibiotics to prevent group B streptococcal disease.

Scope and outline of the investigations.

The objective of the investigations in this thesis was to characterize the pharmacokinetics of antibiotics in the prevention of group B streptococcal (GBS) infections in pregnancy with emphasis on the possible changes which may occur in the perinatal period. A specific objective was to assess the implications of potentially altered maternal pharmacokinetics on the exposure of the infant.

Group B streptococcus (GBS, *Streptococcus agalactiae*) has been known as a human pathogen since 1938[1]. It is a gram-positive coccus, growing in chains or as diplococci. Because GBS causes complete destruction of red blood cells on sheep blood agar, colonies produce a characteristic appearance with narrow surrounding zone of β-hemolysis. Based on the expression of antigenic capsular carbohydrates, GBS is classified into nine serotypes: Ia, Ib and II-VIII. Recently a tenth serotype has been proposed[2]. Like carbohydrates, proteins are also expressed on the bacterial surface. Differences in the expression of carbohydrates and surface proteins account for differences in the pathogenesis of infection and possibly clinical presentation[3,4]. Factors playing a role in the development of invasive infection have been elucidated to some extent[3].

In pregnancy GBS may cause a variety of serious infections in both mother and neonate. Most commonly known is neonatal GBS disease. GBS disease in the neonate is classified according to the age at which the first symptoms occur. Early-onset GBS disease (GBS-EOD) presents within the first week of life and late-onset disease (GBS-LOD) presents from 7 to 90 days of life. GBS is a major cause of neonatal morbidity and mortality. Diseases caused by GBS include sepsis, pneumonia and meningitis. GBS-EOD is usually acquired during delivery by neonates born from mothers colonized with GBS in the rectovaginal tract. Up to 35% of pregnant women is colonized with GBS in the rectovaginal tract, most often without having symptoms[5,6]. Fortunately, only 1% of neonates of colonized mothers develop GBS-EOD. The occurrence of maternal GBS infections is often disregarded.

Intravenous administration of antibiotics is now the cornerstone of the prevention of GBS-EOD and of the treatment of intra-amniotic infection during pregnancy, shortly before and during labor. During pregnancy, antibiotics are administered intravenously to the mother, with the fetus as actual target of prophylaxis of neonatal GBS disease. Antibiotics are administered as short infusions and reach the fetus after transplacental transport. To protect both mother and neonate from GBS infections, the concentration-time profiles of the prescribed antibiotics have to be adequate in both maternal and fetal serum. A limitation of the current dosing regimens as recommended by the Centers of Disease Control and Prevention (CDC) is that they are not evidence-based in the sense that the actual exposure profiles have not been determined.

To study the efficacy of the recommended dosing regimens for GBS-EOD prevention, knowledge of the disposition of drugs in the body is necessary.

Pharmacology (Greek 'φαρμακος' for medicine or drug and 'λογος' for study) is the science of the interactions of chemicals with the human body[7]. These interactions are divided into two classes: pharmacokinetics (PK) and pharmacodynamics (PD). Pharmacokinetics is the study of how the body absorbs, distributes, metabolizes and excretes drugs. The calculation of various rates at which these processes occur brings a quantitative component to assessing drug action[8]. PD is the study of the biochemical and physiological effects of drugs, the mechanisms of drug action and the relationships between drug concentration and effect. The effects of drugs are related to the time course of the drug concentration in plasma, albeit that these relationships may be complex[9,10].

To describe the pharmacokinetics of a specific drug in a target patient group, it is important to take inter-individual variability into account. To this end advanced data-analysis techniques such as non-linear mixed effects modeling are increasingly applied. This is often referred to as the "population approach". Using population pharmacokinetics the data of a group of individuals is simultaneously analyzed and the sources and correlates of variability in drug concentrations among individuals are studied. Specifically, in this manner patient demographical and therapeutical characteristics which might influence the pharmacokinetics are included in the analysis as covariate(s). A further advantage is that the population approach allows the analysis of data from unbalanced study groups[11,12]. This is particularly important for pharmacokinetic studies in pregnant women, because blood sampling might be limited due to practical and emotional problems. Typically, the number of blood samples collected from pregnant women during labor will be less compared to women before onset of labor. The number of umbilical cord blood samples is even more limited, because they can be collected only once for each patient. Thus, population pharmacokinetics describes the pharmacokinetics of a population of subjects. Furthermore, it tries to identify in a quantitative manner the factors that influence the pharmacokinetics. As such population pharmacokinetics constitutes a basis to adjust drug dosages in specific patient populations.

The description of the pharmacokinetics obtained in the population analysis, can be used to evaluate the efficacy of therapy and to optimize dosing regimens. Monte Carlo Simulation (MCS) is a technique used to evaluate the probability of achieving therapeutic concentrations using different dosing regimens. MCS is performed using pharmacokinetic parameters, data on the parameters describing the drug concentration-effect relationship and data on the inter-individual variability in these parameters[13-18]. To study the efficacy of antibiotics, the susceptibility of the micro-organisms is of importance. The susceptibility of bacteria is indicated by the Minimum Inhibitory Concentration (MIC). For the antibiotics used in the prevention of GBS-EOD, the efficacy is determined by the time the antibiotic concentration exceeds the MIC (time-dependent mechanism of action)[19-21].

The aim of the research presented in this thesis was to describe the pharmacokinetics of the antibiotics used in the prevention of GBS-EOD. Preventing GBS-EOD requires an adequate concentration-time profile in both the mother and the fetus. To this end the effects of various dosing regimens and inaccuracies in the antibiotic administration on the efficacy of the amoxicillin were evaluated. Finally after birth, adequate dosing in (preterm) neonates with a suspected infection is essential. This requires knowledge of the pharmacokinetics in neonates. In this respect the pharmacokinetics of penicillin G in neonates was studies as well.

Part I of this thesis reviews background information on the prevention of GBS-EOD and maternal GBS infections. In **chapter 2** a detailed review of the use of antibiotics in the era of the prevention of GBS-EOD is presented. The available evidence on the pharmacokinetics of antibiotics used as intrapartum prophylaxis in relation to infection parameters and GBS-EOD incidence is described, to evaluate the efficacy and safety of currently advised prophylaxis. The efficacy of the prophylaxis is mainly attributed to a lowering of the incidence figures of GBS-EOD. However, incidence figures are influenced by many factors and may therefore not be considered conclusive proof. The available data on the changes in incidence figures, as well as data on the interruption of vertical transmission of GBS carriage, support the idea that antibiotics prevent GBS-EOD. But to advise antibiotic prophylaxis to approximately 35% of all pregnant women during labor, more data are needed on the pharmacokinetics of the antibiotics and on the unintended consequences for both mother and neonate.

Guidelines on the prevention of GBS disease focus on infections of the fetus. The fact that GBS also causes infections in pregnant women is less appreciated. Various maternal GBS infections, their characteristics, associated neonatal morbidity, and prevention and treatment strategies during pregnancy, delivery, and in the postpartum period are reviewed in **chapter 3**. GBS infections in the mother cause less morbidity than neonatal infection, but occur more commonly. Especially during the course of pregnancy and labor, GBS can endanger both mother and the fetus. Postpartum mastitis can also threat mother and the neonate, because it may be a cause of late-onset or recurrent neonatal GBS disease. With early recognition and proper treatment, maternal and neonatal severe morbidity and mortality due to GBS infections are rare.

The penicillins, such as amoxicillin, are antibiotics of first choice in the prevention of GBS-EOD during pregnancy and delivery. As an alternative, clindamycin is used. In **part II** the pharmacokinetics of amoxicillin is described. It is used as prototype to study all issues related to the prevention of GBS infection in pregnant women. In **chapter 4** the pharmacokinetics of amoxicillin in pregnant women with preterm premature rupture of the membranes (PPROM) is described. Pharmacokinetic parameter estimates for patients with PPROM were all within the ranges reported in the literature for healthy non-pregnant individuals. **Chapter 5**

focuses on the influence of labor on the pharmacokinetics of amoxicillin. To this end the pharmacokinetics were determined in patients before the onset of labor, during labor and in the immediate postpartum period. An effect of labor was seen on the peripheral volume of distribution. A decrease in the peripheral volume of distribution was seen during labor and even more in the immediate postpartum period. In case of suspected intra-amniotic infection, co-amoxiclav, a combination of amoxicillin and clavulanic acid is used. When drugs are administered simultaneously, there is a possibility that these drugs influence their pharmacokinetic behavior. The influence of co-administration of clavulanic acid on the pharmacokinetics of amoxicillin is presented in **chapter 6**. In agreement with observations from earlier studies in healthy subjects it is shown that clavulanic acid has no effect on the pharmacokinetics of amoxicillin in pregnant women. Because the fetus is the actual target of the prophylaxis, the transfer of drugs over the placenta is an important factor. The investigations in **chapter 7** aim therefore characterization of the concentrations of amoxicillin in umbilical cord serum and neonatal serum in relation to the concentrations in maternal serum. Approximately 1 hour after the start of the intravenous administration 2 gram amoxicillin over 30 min to the mother the neonatal concentration reached its highest level, and thereafter exceeded the concentrations in venous umbilical cord blood. Finally the population model of the amoxicillin pharmacokinetics in pregnant women with PPROM was used in **chapter 8** to evaluate the probability of target attainment (as indicated by the MIC) after various dosing regimens and inaccuracies in the administration of the amoxicillin using Monte Carlo Simulations. Both regimens recommended by the CDC as well as the regimen described in the Cochrane Library result in adequate maternal concentration-time profiles[22,23].

Most patients included in our study were relatively healthy. To describe the influence that co-morbidity might have on the pharmacokinetics we present a case-report of a pregnant women with PPROM and severe vomiting in **chapter 9**. We hypothized that the extreme vomiting had resulted in additional physiological changes and thereby changing the distribution of the amoxicillin.

In **part III** pharmacokinetics of other antibiotics used in prevention or treatment of GBS infections are presented. Patients allergic to penicillins may not be treated with amoxicillin and in this condition clindamycin is used instead. Moreover in patients who need endocarditis prophylaxis, clindamycin is prescribed as well. Clindamycin should be studied in a similar manner as described for amoxicillin, but limited data were available. In **chapter 10** the pharmacokinetics of clindamycin in pregnant women is described. A limited number of patients was available to study the pharmacokinetics in pregnant women and the transfer over the placental barrier. The results of our preliminary investigations show that for the average pregnant women the recommended dosing regimen is adequate, but it is doubtful whether the concentrations in the fetus are also adequate.

Despite the prophylactic measures, neonates may still acquire GBS-EOD, partly because mothers of these neonates were not selected for antibiotic prophylaxis. In some neonates an overwhelming intra-amniotic infection had already developed at the time the antibiotics are prescribed. In both occasions, neonates have to be treated with antibiotics after birth. In this respect it is important that especially, preterm neonates are vulnerable for the development of GBS-EOD. For several drugs the pharmacokinetics in preterm neonates has been found to be different from older children and adults[24-26]. In **chapter 11** therefore, the pharmacokinetics of penicillin G in very preterm neonates is described. The pharmacokinetics in neonates with a gestational age of less than 32 weeks differs from that in adults and older infants, which is indicated by a prolonged terminal half-life.

In the general discussion (**part IV, chapter 12**) all results of the various investigations are discussed and future perspectives are presented.

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Chapter 2

Antibiotics in the prevention of neonatal group B streptococcal infections: the evidence.

Anouk E. Muller, Rob A. Voskuyl, Paul M. Oostvogel, Lia (C.) Liefaard, Eric A.P. Steegers, Johan W. Mouton, P. Joep Dörr

Abstract

To prevent group B streptococcal early-onset disease (GBS-EOD) in the neonate, many pregnant women are treated with antibiotics during labor and/ or delivery. During the last years several countries implemented the screening-based strategy to prevent GBS-EOD, resulting in an increase in the use of antibiotics during delivery. Overall, most incidence figures of culture-proven GBS-EOD decreased in the last decades. Because incidence figures are influenced by multiple factors, a decrease cannot be considered as exclusive evidence for efficacy of antibiotics. Despite limited knowledge on the efficacy of the antibiotics prescribed, they are used worldwide as preventive measure in up to 35% of pregnant women shortly before and during delivery. In this paper, we review the available evidence, from pharmacokinetics of antibiotics used as intrapartum prophylaxis to infection parameters and GBS-EOD incidence figures to evaluate the efficacy and safety of currently advised prophylaxis.

Introduction

In the 1970s group B streptococcus (GBS) infection emerged as a major cause of neonatal morbidity and mortality in the industrialized world. This led the Centers for Disease Control and Prevention (CDC) and other organizations to issue guidelines in the 1990s for prevention of neonatal GBS disease by intrapartum prophylaxis with antibiotics (IPA)[1]. CDC guidelines discuss a wide variety of issues associated with GBS disease, such as transmission of infection and selection of patients eligible for prophylaxis, and highlight a steady decline in incidence of GBS disease since introduction of IPA. However, the guidelines recommend regimens for IPA, but give no arguments for dose, dosing interval and duration of treatment[1,2].

The decline in incidence of GBS early-onset disease (GBS-EOD), i.e. onset of symptoms within 7 days after birth, has generally been considered as evidence for effectiveness of IPA. However, because other factors may have influenced incidence figures as well, it is arguable whether this is conclusive proof. In a recent review it was discussed why time-trend analyses have their drawbacks in this respect[3]. The question remains how else efficacy should be judged and whether available evidence justifies widespread IPA.

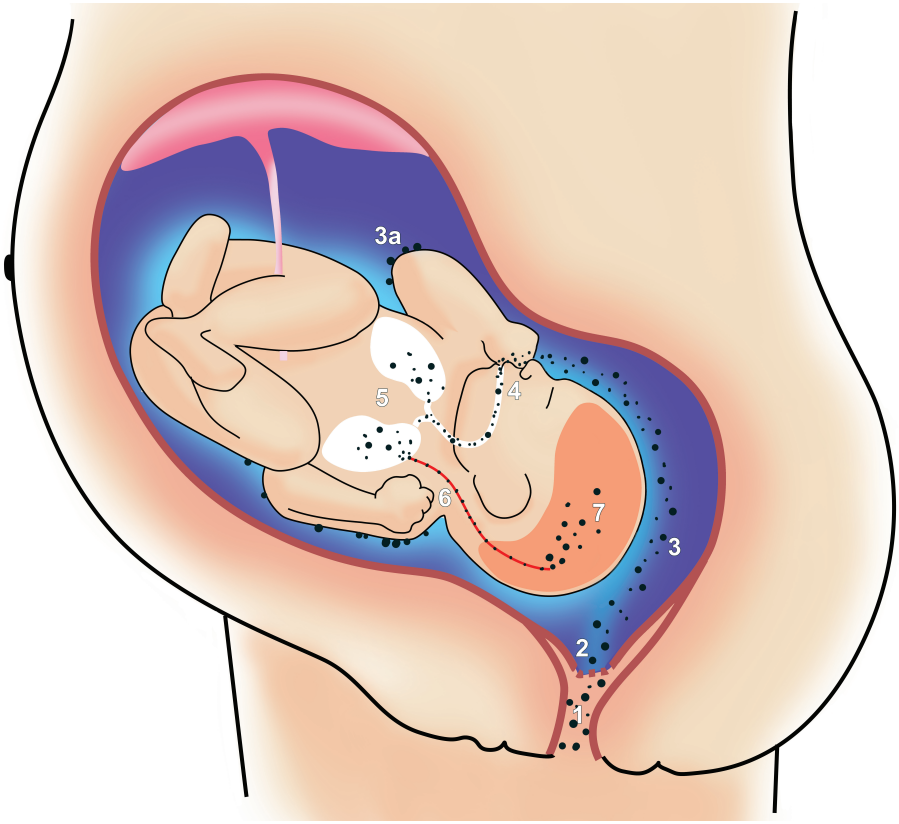
This paper reviews the available evidence for efficacy of IPA used in the prevention of neonatal GBS disease. We will first describe the clinical presentation of GBS-EOD, etiology and the working mechanisms of the recommended antibiotics. Pharmacokinetic-pharmacodynamic measures generally used to predict efficacy of antibiotic therapy, as well as the available data during pregnancy and delivery are described. Clinical studies were also reviewed to determine the likeliness for efficacy of IPA from this point of view. Finally, unintended consequences for mother and neonate are discussed.

Search strategy and selection criteria

Data for this review were identified by searches of PubMed, MEDLINE, Current Contents, the Cochrane Library, and references from relevant articles. Search terms included combinations of “Streptococcus agalactiae”, “group B streptococcus”, “pharmacokinetics”, “pharmacodynamics”, “elimination”, “half life”, “incidence”, “epidemiology”, “vertical transmission”, “neonatal”, “fetal”, “amniotic fluid”, “colonisation”, “anaphylaxis”, “adverse reactions”, “immune system”, and terms for the specific antibiotics (eg, “penicillin”). No date or language restrictions were set in these searches, but only English, Dutch, German, French and Spanish manuscripts were selected afterwards. No studies were excluded based on study design.

Etiology and clinical presentation of neonatal GBS disease

In figure 1 it is illustrated how GBS-EOD is usually acquired during labor or delivery. Of neonates born from GBS colonized mothers 1-2% develop GBS-EOD[1]. Mortality has decreased in the few last decades, but survivors may suffer from severe disability (e.g., hearing or visual loss, uncontrolled seizures, impaired psychomotor development and/ or mental retardation)[1].



Designed by Vincent Khouw (VMK designs)

***Figure 1**: Hypothesized pathogenesis of GBS-EOD.*

1 Colonization in the rectovaginal compartment; 2 Rupture of the membranes; 3 GBS enters the amniotic fluid; 3a GBS colonization of skin and mucocutaneous areas; 4 Aspiration of infected amniotic fluid; 5 Infected amniotic fluid causes pneumonia (if the bacterial load is high enough); 6 Entry of GBS in the bloodstream (sepsis or bacteremia); 7 Entry in cerebrospinal fluid after hematogenous spread (meningitis). (See color inlay for a full color version of this figure.)

GBS meningitis results in children with disabilities in 34.8% of cases[4], and in an earlier study 33% of the surviving children showed abnormalities related to GBS septicemia or meningitis[5].

GBS-EOD may present in different ways. GBS-EOD is diagnosed as culture-proven when streptococci are isolated from blood and/or cerebrospinal fluid and when physical signs and laboratory results are clear. The diagnosis probable GBS-EOD is used for cases of serious neonatal disease when GBS is detected at various sites, but not in blood and/or cerebrospinal fluid[6]. Finally, culture-proven GBS-EOD may also present as "asymptomatic" bacteremia[7-9]. Asymptomatic bacteremia was defined as positive blood cultures for GBS in neonates without clinical signs of infection. Such cases may be discovered by accident when blood cultures are taken shortly after birth in neonates when only maternal risk factors were present during delivery[2]. In this way, culture-proven GBS-EOD was found to be asymptomatic in 4-20%[10-15]. Weisman et al found that 15% of 149 term neonates with bacteremia were asymptomatic, whereas all 96 preterm neonates had clinical or laboratory signs of infection[7]. It is unclear for how long bacteremia persists. Prolonged bacteremia for >24 hours has been described[8]. The possibility of developing a life-threatening illness and poorly understood pathogenesis of late-onset GBS disease (GBS-LOD, i.e. 7-90 days of life), justifies treatment of asymptomatic GBS bacteremia in neonates.

Both host related factors and bacterial properties may increase the risk on GBS-EOD. Host related factors include >18 hours of ruptured membranes, fever during delivery, GBS bacteriuria in current pregnancy, having a neonate with GBS disease in obstetrical history and preterm delivery. These factors are associated with an increased risk on GBS-EOD and were incorporated in guidelines to prevent GBS-EOD[1]. At least one of the risk factors preterm delivery, intrapartum fever and membrane rupture of at least 18 hours is found in 49% of GBS-EOD cases[16]. On the other hand, bacterial virulence properties might also influence this risk[17]. But, unfortunately little is known about specific virulent GBS subtypes. Furthermore, there are some other risk factors for GBS-EOD known, such as low levels of maternal anticapsular antibodies, increased number of vaginal exams and intrauterine fetal monitoring[16,18,19].

Intrapartum prophylaxis with antibiotics

The primary aim of IPA is to prevent fetal infection by lowering the bacterial load sufficiently. Optimal IPA requires an antibiotic that preferably selectively kills GBS. The ideal dosing regimen should be designed in a way to achieve prompt, effective concentrations at the site of infection for sufficient time to lower the bacterial load to a harmless level. At the same time toxicity for both mother and fetus should be avoided.

Different strategies have been used to select candidates for IPA[1,2]. For instance, current CDC guidelines[2] advise the administration of intrapartum antibiotics to all GBS carriers during delivery (2-35% of all pregnant women[20-27]). Dosing schedules are shown in table 1. Women with unknown GBS carriage during delivery, are treated with IPA when host related risk factors are present[2].

Dosing regimen (CDC)[2]

Antibiotic	Initial dose	Subsequent dose	Dosing interval	Patients
Benzyl-penicillin	5 million Units *	2.5 million Units *	4 h	Not penicillin allergic
Ampicillin	2g	1g	4 h	Not penicillin allergic
Cefazolin	2g	1g	8 h	Allergic to penicillin; low risk of anaphylaxis
Clindamycin	-	900 mg	8 h	Allergic to penicillin; high risk of anaphylaxis; susceptibility to clindamycin proven
Erythromycin	-	600 mg	6 h	Allergic to penicillin; high risk of anaphylaxis; susceptibility to erythromycin proven
Vancomycin	-	1g	12 h	Allergic to penicillin; high risk of anaphylaxis; resistant to clindamycin and erythromycin

* Dutch and Australian guidelines deviate without an explanation from these guidelines, advising a initial dose of 2 million Units and subsequent doses of 1 million Units[115].

***Table 1**: Dosing regimen as recommended by CDC[2]*

The choice of the antibiotics

Antibiotics as recommended by CDC are active against GBS. Beta-lactam antibiotics, such as the penicillins and cephalosporins, are active against GBS by disrupting the synthesis of the cell wall. Both erythromycin and clindamycin show a growth-inhibiting action by interfering with protein synthesis. Vancomycin resembles the penicillins with regard to the mechanism of action in that it interferes with cell wall synthesis and thereby increases cell wall permeability.

According to the guidelines[2], penicillin G is the antibiotic of first choice because of its narrow spectrum and lack of resistance of GBS to penicillin G, with ampicillin as alternative. Although not mentioned in the CDC guidelines, amoxicillin could be used as well. GBS may or may not be cross resistant to erythromycin and clindamycin, depending on the mechanism of resistance, and this is becoming an increasing problem[28-31]. Vancomycin is the last resort, and is not optimal because of its low intrinsic killing activity.

Adequate concentrations

Considering the infection pathway (figure 1), adequate levels in both fetal serum and amniotic fluid (AF) are required. Measures to evaluate efficacy should ideally concern fetal serum and amniotic fluid levels. Maternal levels are a prerequisite for adequate fetal serum levels and eliquibrium between fetal and maternal levels is reached within limited amount of time. Therefore, maternal concentration-time profiles might be used as well. Before reviewing and evaluating available data, we will discuss general criteria for effective concentrations and issues related to pregnancy which might influence pharmacokinetics and pharmacodynamics.

General pharmacokinetic-pharmacodynamic measures for efficacy

In the simplest approach antibiotics are divided into agents that display time-dependent killing and agents with a concentration-dependent action. While this concept is still valid, it should be noted that several more refined measures have been developed in pre-clinical studies which are predictive for efficacy in humans as well[32].

Antibiotics mentioned in the CDC guidelines[2], except clindamycin and vancomycin, display time-dependent killing[32]. For beta-lactam agents the time the free fraction of the drug, i.e. the fraction not bound to proteins (*f*), above the minimum inhibitory concentration (MIC) is the best predictor for efficacy (*f*T>MIC)[33-36]. In general, a *f*T>MIC for 30-50% of the dosing-interval is considered adequate for optimal treatment in non-neutropenic patients[34,37,38]. Clindamycin also has a time-dependent action in vitro, but clinical efficacy is more closely related to the area under the concentration curve over MIC for 24 hours ($AUC_{0\text{-}24h}$/MIC)[32]. Similarly, although vancomycin displays time-dependent action in in vitro studies, animal and clinical studies suggest that the effect of vancomycin is better related to the $fAUC_{0\text{-}24h}$/MIC ratio[33]. Furthermore, vancomycin seems to display some concentration-

dependency. For this drug studies indicate that the *f*AUC$_{0\text{-}24h}$/MIC ratio needs to be at least 25-30 to be effective[34,37], but may be much higher for optimal effect.

Development of invasive disease is also dependent on interaction of GBS with the neonatal immune system. Three basic mechanisms are required for effective elimination of invasive GBS: chemotaxis, phagocytosis and intracellular as well as extracellular bacterial killing. Deficiencies on all levels have been identified in neonates, particularly in those born prematurily[39-41]. Infants are protected in part by active transplacental acquisition of maternal antibodies that significantly occurs in the third trimester of pregnancy[42]. The neonatal adaptive immune system is still poorly developed due to low synthesis of IgG, especially of prematures[42]. Deficiencies in both innate and adaptive immune system make neonates vulnerable for GBS-EOD. Therefore (premature) neonates have to be regarded as immunocompromised patients and consequently the *f*T>MIC should be larger (40-60%) than in immunocompetent patients[38].

Pharmacokinetics in the maternal-fetal unit

Since antibiotics reach the fetus after transplacental transfer, adequate maternal serum levels are the first requirement to reach fetal serum concentrations. Secondly, antibiotics should reach the AF. Transplacental passage of antibiotics occurs primarily by simple diffusion of the free fraction[43]. Therefore, the rate of transfer is related to the maternal-fetal concentration gradient and is inversely proportional to the thickness of the placental membrane[44]. The thickness decreases with gestational age and in various disease states, like diabetes and hypertensive disorders complicating pregnancy[45]. In the third trimester AF levels of antibiotics eliminated by the kidneys largely depend on fetal renal excretion[46,47] and are influenced by maturation of the fetal kidneys[48].

The continuously changing physiological adaptations to advancing pregnancy are likely to modify the pharmacokinetics. Therefore, to determine whether the recommended dosing regimens are indeed adequate to achieve the desired concentration profiles it is essential to focus on pharmacokinetic information obtained shortly before and during labor. Unfortunately, most studies reviewed in Table 2 present data that are far from optimal to make a sound judgement. Essential data are often missing (e.g. fetal concentrations, protein binding) and presented pharmacokinetic data or observed concentrations do not allow proper estimation of *f*T>MIC or other indices. Also, the numbers of patients included per study were limited and exhibited a wide range in gestational ages. Furthermore, most studies included patients without uterine contractions, a factor which might further influence pharmacokinetics[49,50].

CDC guidelines call for at least 4h of IPA prior to delivery to be adequate[2]. From a pharmacokinetic point of view there is no rationale for this interval of 4 hours. Antibiotics reach fetal serum within several minutes after the administration to the

Antibiotic	Maternal serum levels	Total body clearance	Terminal half life	Cord blood levels	Amniotic fluid levels	References
Benzylpenicillin	Adequate	Increased	Decreased	-	-	116
Ampicillin	Decreased*	Increased*	Decreased*	Detectable after 3-10 min; equal to maternal serum after 2h. Above MIC for GBS 27min-8h after iv injection (1g).	Below MIC for GBS within the first 30-67 min	48-50,62,117-123
Cefazolin	Decreased	Increased	Decreased	Above MIC for GBS 0.5-6h after iv injection	Above MIC for GBS 0.5-6h after iv injection	124,125
Erythromycin	Decreased	-	-	2-10% of maternal levels (one study 5-20%)	-	126-132
Clindamycin	Unchanged	-	Slightly decreased	50% of maternal levels	First 30-60 min after iv injection to the mother not detectable	121,126,128, 132,133
Vancomycin	-	-	Similar**	Feto-maternal ratio 0.76**	-	134

* Voigt et al. found the pharmacokinetics in pregnant women to be similar to non-pregnant women. But being in labor affected the pharmacokinetics significantly[49].

** Data derived from one patient in the second trimester of pregnancy.

***Table 2**: pharmacokinetic data of the antibiotics recommended in CDC-guidelines.*

mother[48]. Afterwards the concentration will decrease both in fetal and in neonatal serum. Antibiotics in neonatal serum will continue to eliminate bacteria with the same rate as before birth. Therefore, a short time interval between administration of antibiotics and delivery does not reduce adequacy of IPA.

In conclusion, these data, especially data in relation to pharmacodynamic indices, are insufficient to judge efficacy of IPA. Most information is based on concentrations in maternal serum, but antibiotics reach fetal serum after transfer across the placental barrier, what might influence the T>MIC. Pharmacokinetic-pharmacodynamic measures found for other infections in non-pregnant individuals or animal models are not necessary valid in fetal serum and amniotic fluid.

Clinical evidence in favor of the efficacy of IPA

In addition to pharmacokinetic data other studies may contribute to the evaluation of efficacy of GBS-IPA. The most direct indicator for efficacy is the bacterial load in neonatal blood cultures. Also the number of colonized mucocutaneous areas in the neonate has been shown to be a determinant of GBS-EOD[51]. Neonates with GBS-EOD had significantly more GBS positive surface areas than infants without GBS-EOD[51]. Obviously, effective prophylaxis should be reflected in decreased incidence figures. However other factors may influence these figures as well and blood cultures are taken from a selection of the neonates. Therefore, it is important to separate the contribution of IPA from the contribution of other factors whenever possible.

Blood cultures

Prospective studies comparing antibiotic treatment with no treatment provide the strongest evidence for effectiveness. In a randomized prospective study Boyer and Gotoff[52] observed a lower incidence of positive blood cultures in neonates of GBS carriers treated with ampicillin intrapartum compared to neonates of patients not treated with ampicillin[52]. In contrast to the present guidelines[2], neonates in the study of Boyer received antibiotics after maternal IPA as well[52]. The reduced number of positive blood cultures suggests that IPA decreases the incidence of GBS-EOD[52], but since clinical neonatal outcome was not reported, it cannot be concluded that current IPA is optimal.

Another issue is apparent IPA failure. Six studies report that 6-19% of neonates with invasive GBS disease were born from mothers with IPA[12,14,53-57]. Obviously, antibiotic treatment was not optimal in these cases. Maternal fever is associated with the presence of positive neonatal blood cultures after IPA (referred to as prophylaxis-failure)[12,54,58]. Most likely adequate fetal serum levels are achieved within the first hour, but apparently more time is needed to eradicate GBS, as is

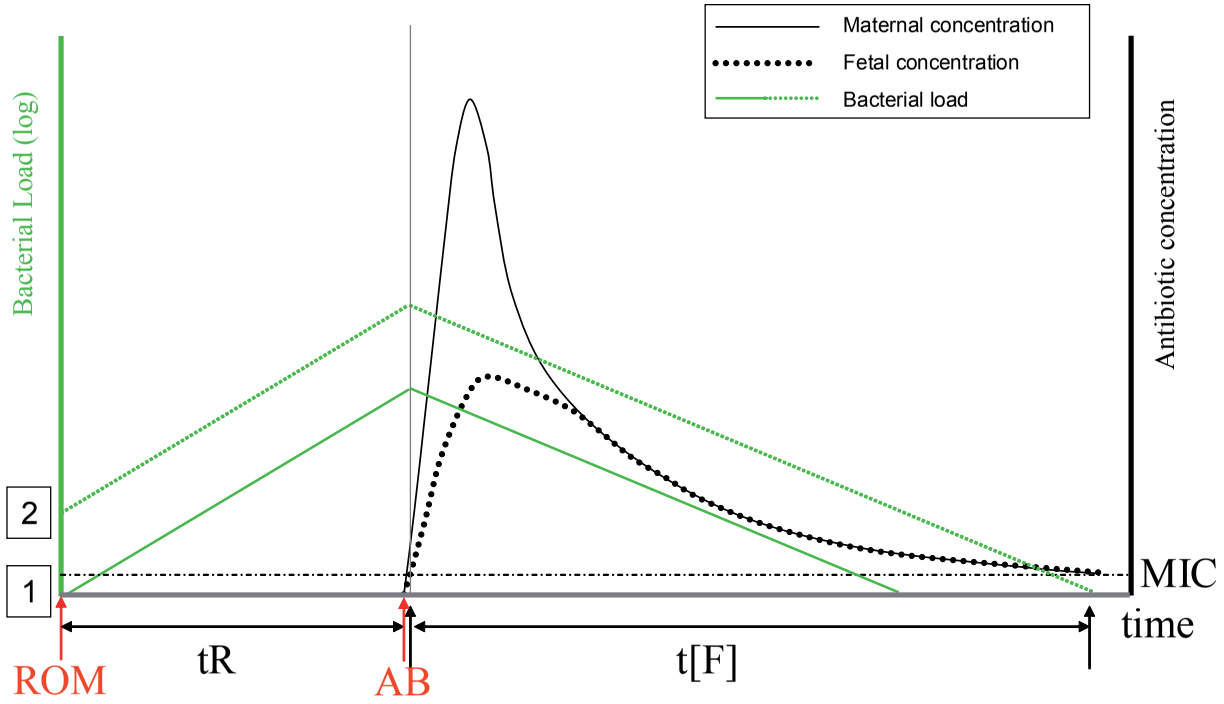


Figure 2: *The effect of antibiotic prophylaxis on the bacterial load of GBS.*

ROM= Rupture of membranes, tR= time between ROM and start of antibiotics, AB=start of administration of antibiotic, MIC=minimum inhibitory concentration, t[F]= time the fetal concentration exceeds the MIC; 1 changes in bacterial load. 2 enhanced bacterial load in patients in maternal fever or prolonged ROM. (See color inlay for a full color version of this figure.)

illustrated in figure 2. The amount of time needed to eradicate GBS depends on the bacterial load. The bacterial load is believed to be increased in cases with maternal fever and prolonged rupture of the membranes. Consequently, a short time span between administration of IPA and delivery will result in a higher number of positive blood cultures taken immediately after birth. This is consistent with the fact that within cases of prophylaxis-failures duration of IPA <1-2 hour prior to delivery is indicated as risk factor for failure[53,56,59]. This indicates that IPA is insufficient when an overwhelming infection might have been established in utero before initiation of antibiotics, because the antibiotic concentration was maintained for insufficient time to eradicate all bacteria at the time the blood culture was taken.

Reduction in neonatal colonisation

As explained above, observational studies suggested that vertical transmission of GBS might be interrupted by IPA. Most often neonatal colonization is determined by the use of cultures taken from three mucocutaneous areas (pharynx, umbilicus, and external auditory canal) to serve as a measure of effectiveness. In the absence of IPA in vaginal deliveries, neonates born from GBS colonized mothers were colonized in 43% to 53% at one or more surface areas[60-64]. Transmission from mother to child after caesarean section in patients with ruptured membranes or active labor was 25.9%[61]. After administration of IPA with ampicillin a lower neonatal colonization rate has been seen after vaginal delivery, varying from 0% to 10%[52,60,62,64-66].

The time interval between administration of IPA and delivery is an important determinant in interrupting mother-to-child transmission (figure 2)[63, 65]. Adequate AF antibiotic concentrations are likely to be involved in eradication of GBS from surface areas. Since there is some time needed to achieve adequate AF concentrations and eradicate GBS from these areas, the bacterial load will decrease after an increased time interval between IPA and delivery. De Cueto et al. found for ampicillin that when this interval is at least 2 hours, vertical transmission of GBS was minimized to 1.5%[63].

Unfortunately, data on vertical transmission after IPA with clindamycin, erythromycin or vancomycin are scarce. One study in 7 patients receiving intramuscular erythromycin for an unknown period demonstrated that none of the neonates carried GBS when cultured within 24 hours after birth[67]. Since adequate AF antibiotic levels, rather that adequate fetal serum concentrations are likely to be involved in eradication of GBS from mucocutaneous areas of the fetus, the effect of IPA with erythromycin and clindamycin might be limited or delayed.

Changes in incidence figures and case fatality

Most trends in incidence rates of culture-proven GBS-EOD decreased within a geographical area after implementation of IPA, suggesting a causal relation. Before the implementation of prophylaxis in the US the incidence of GBS-EOD fell from 2-3/1000 live births in the 1970s-1980s to 1.4-1.8/1000 live births in 1990 with a constant prevalence of maternal GBS colonisation of 20-25%[68]. While it is likely that IPA is in part responsible for the decrease in incidence and low mortality rate, other factors may contribute as well, among which are early recognition of infection and improved neonatal care[3,56]. Furthermore, natural fluctuations in incidence figures of GBS-EOD as large as 2.85 to 0.45 per 1000 live births from year to year[69] may occur within regional populations and may therefore erroneously be interpreted as being caused by IPA. Such fluctuations may be due to changes in prevalence of maternal colonisation as well as variation in GBS subtype distribution.

There are other issues to be considered as well. Many studies on incidence are difficult to compare because of methodological diversity[56,70,71]. Since there is an extremely low risk for full-term infants born by elective caesarean section without rupture of the membranes or onset of labor on GBS-EOD[2,72], an increased application of this procedure will decrease the incidence. Incidence figures should therefore be corrected for this aspect.

The substantial decline in incidence figures is based on data of culture-proven GBS-EOD. Since suboptimal IPA may lead to negative blood cultures in clinically ill neonates (see above), studies can be interpreted with confidence only when incidence figures of culture-proven as well as probable EOD are reported[57, 73]. With suboptimal IPA the incidence of culture-proven EOD will be decreased, while the incidence of probable EOD might be increased. Estimates of the incidence of probable GBS-EOD are higher than culture-proven incidence rates, indicating a greater disease burden than suggested by studies based on only culture-proven GBS-EOD[57,74,75]. Comparing incidence rates after correction for underreporting before and after the introduction of IPA showed that the incidence of probable GBS-EOD was constant in the Netherlands (1.3-1.4/1000 live births). There was only a limited decrease in the culture-proven GBS-EOD from 0.54/1000 live births before introduction of IPA to 0.36/1000 live births afterwards[75]. Because asymptomatic bacteremic neonates are often included the culture-proven incidence rates, local protocols on neonatal blood cultures can also influence incidence figures.

Finally, incidence figures do not always decline. Noteworthy is the unchanged incidence of GBS-EOD in a hospital where the intrapartum use of antibiotics increased from 13% to 44% of all deliveries between 1989 and 2002[76]. As reviewed by Gilbert[3] earlier, it appears from these findings that incidence rates provide only moderate evidence for efficacy of the GBS prophylaxis.

In the last 40 years the case fatality rate of culture-proven cases of GBS-EOD has decreased from 55% in the 1970s to <10% in 2000-2005[2,53,57,77]. An important

factor in this decline is likely to be the improved neonatal care. IPA may have contributed to this decline by advancing the antibiotic effect on the child and decreasing the severity of the disease. However, recently Norway reported an yet unexplained, marked increase in case fatality from an average of 5.8% in 2000-2005 to 33% in the first 6 months of 2006 and a slightly increased incidence of invasive GBS disease in neonates in the first 90 days of life using a risk factor based strategy[78]. Such changes in case fatality rates without alterations in antibiotic policy, might be explained by changes in the virulence characteristics of circulating GBS strains.

From these clinical studies we can conclude that the intrapartum administration of antibiotics to prevent GBS-EOD is likely to have clinical effect. However, it is unclear to what extend the decrease in GBS-EOD can be attributed to the administration of antibiotics and whether the currently used dosing schedules are optimal. After reviewing the positive effects of IPA, it is also important to discuss the unintended consequences of IPA, as will be described in the following.

Unintended consequences of IPA

To justify the administration of antibiotics as prophylaxis against GBS-EOD in up to 35% of women in labor, apart from being effective, prophylaxis should have minimal risks for both mother and child. The risk for the mother is limited to the risk on anaphylactic reactions on administered antibiotics. The (long-term) unintended consequences for neonates are still under debate. An increase use of (suboptimal) IPA may also affect the susceptibility of GBS.

Maternal risks

An increased use of antibiotics will result in more adverse reactions. The most serious reaction is an anaphylactic shock with consequences for both mother and fetus. Many pregnant women have a history of penicillin "allergy", often described as a rash. In spite of the fact that most antibiotic-associated rashes are not IgE-mediated, the risk of anaphylaxis can not be ignored[79]. In general, the incidence of anaphylaxis among inpatients has been reported to be three to five per 10,000[80]. The incidence of anaphylaxis after administration of penicillin is estimated to be 0.01% with a mortality rate of 9%[81]. Anaphylaxis occurs more often after parenteral administration than after oral administration. In pregnant patients with anaphylactic shock there will be fetal distress due to maternal hypoxia and hypotension. On the other hand parenteral antibiotics used for GBS prophylaxis have rarely been noted to cause severe reactions in pregnant women without a history of penicillin allergy[82-85]. Penicillin skin testing can be performed in advance in pregnant women and penicillin can be administered safely if the result is negative[79].

Neonatal risks

Several possible unintended consequences of IPA have raised concern for the neonate. Firstly, some investigators have reported an increase in incidence of non-GBS-EOD. These increases appear to be limited to preterm or low-birth-weight infants and ampicillin-resistant pathogens[86-88]. Among cases of sepsis, non-GBS sepsis in infants was caused more frequently by ampicillin-resistant pathogens in the era of IPA[89,90]. Especially rates of ampicillin-resistant *Escherichia coli* sepsis have increased among preterm neonates[86,91,92]. Is was suggested that the increase of ampicillin-resistant pathogens might be partially attributable to maternal antibiotic exposure before delivery. But, as reviewed by Moore et al., there are some confounders in the interpretation of these studies[93]. None of the studies was designed to estimate the efficacy of IPA against susceptible infections. Duration and indication of IPA as well as the presence of other known risk factors for EOD, like prematurity, and natural fluctuations in incidence numbers should be taken into account in the analysis[93]. Simultaneously, the proportion of community-acquired *E. coli* infections that are ampicillin-resistant has been increasing[94] suggesting that trends in antimicrobial resistance should not be attributed to GBS prophylaxis alone[2]. To summarize, trend analyses do not allow a direct assessment of causality between IPA and risk of non-GBS sepsis[93].

Secondly, one study reported an association between the use of IPA and LOD[95]. GBS-LOD has usually been considered community-acquired. The incidence of GBS-LOD did not change after implementation of the prophylaxis[2]. Glasgow et al. compared the frequency of LOD in infants exposed to IPA and non-exposed infants[95]. Exposure to IPA was strongly associated with the occurrence of LOD. Pathogens causing LOD were more likely to be ampicillin-resistant in infants exposed to IPA. Both findings seemed to be associated to the use of broad-spectrum antibiotics, rather than benzylpenicillin.

There is also some evidence from basic animal and human studies that peripartum antibiotics may have long-term consequences for the neonate. The use of antibiotics during delivery influences the maternal vaginal and fecal flora, which provide the first natural sources of colonizing organisms in the neonatal gut[95,96]. Acquired abnormalities in early-life bacterial colonization may affect the development of the immune system and a change in pattern of initial colonization of the gut in the first days of life may be linked to later development of allergic disease[96,97]. Unlike broad-spectrum antibiotics, benzylpenicillin does not perturb normal gastrointestinal flora[98,99] and for intrapartum amoxicillin the influence was shown to be limited to a reduced initial colonization by *Clostridium* in infants exposed to antibiotics[100]. Apart from IPA it has also been found in infants with an age of one month that the intestinal flora was influenced by such factors as mode of delivery, breast-feeding, hospitalization after birth, prematurity and the presence of older siblings[101].

Notwithstanding the fact that several confounders complicate the interpretation of the relation between IPA and non-GBS sepsis, and that many factors may be involved in the development of the neonatal immune system, the association with IPA may not be ignored. Based on current data, the estimated number of prevented infections from IPA still outweighs the possible neonatal unintended consequences. Concerning the choice of the antibiotic, available data suggest that the risk on neonatal unintended consequences is minimal with the use of benzylpenicillin, compared to broad-spectrum antibiotics, like ampicillin.

Risk on emergence of resistance

Besides aspects of efficacy, dosing schedules should also be designed to minimize the chance of bacterial resistance. Appropriate exposure to antibiotics achieved by adequate dosing is important to limit resistance development[102]. The potential for resistance development can be defined as the ability of a bacterial strain to survive killing and regrow. Thus, there is an inverse relationship between the efficacy of an antibiotic and the resistance induction potential of an antibiotic[103]. For several micro-organisms and antimicrobials the area under the curve of the unbound fraction over MIC (*f*AUC/MIC) has been investigated in the prediction of selecting resistant organisms[104-107]. However, since there are no data on prevention of resistance in GBS, dosing regimens used in the prevention of GBS-EOD can not be judged on their potential to select resistant organisms. Studies in pre-clinical infection models could be very useful for designing dosing regimens that avoid resistance development[32].

Conclusions

Having reviewed data on efficacy of IPA, the question whether IPA is truly preventing GBS infection of the fetus, can not be answered with certainty. Limited available data suggest that IPA to some extent prevents GBS-EOD, but other factors are likely to contribute to lowering of the incidence. Apart from these studies, concerns on unintended consequences for mother and neonate are rising and are still under debate.

It is surprising that discussions on effectiveness of IPA only have concerned proper identification of patients at risk, implementation of the prophylaxis and circumstantial aspects affecting incidence, but have not questioned practice itself. Dosing regimens are based on tradition[108], rather than on pharmacokinetic data during pregnancy. Physiological changes due to complications of pregnancy, such as severe preeclampsia, might also have an additional effect on pharmacokinetics. CDC guidelines call for at least 4h of IPA prior to delivery to be adequate[2]. But neither studies on the decrease of transmission of GBS nor pharmacokinetic data provide a rationale for this 4 hour threshold[109]. Even if a short time interval is

expected between administration of antibiotic and delivery, this is no reason to omit IPA. Of the antibiotics advised for the prevention of GBS-EOD, the penicillins have been studied most extensively. But even their efficacy can not be guaranteed.

The optimal timing in labor to initiate antibiotics is difficult to assess[110]. The presence of risk factors for development of GBS-EOD influences the initiation of prophylaxis. These factors might all be related to an increase in bacterial load in the AF (figure 3). The current practice in GBS prophylaxis is based on the idea that the risk on GBS-EOD primarily exists after rupture of the membranes. Indeed, the bacterial load increases with the duration of ruptured membranes and subsequently the attack rate increases with a marked rise after 18 hours[111]. However penetration of GBS through intact membranes can also occur, leading to severe cases of intra-amniotic infection or abortion[112]. The ability of GBS to attach and invade the chorioamniotic membranes has been demonstrated in vitro[113], but might be limited to a specific GBS subtype. In this scenario it might be appropriate to start antibiotic therapy earlier than is advised now.

Nowadays, many pregnant women are candidates for IPA and this, in conjunction with the lack of high quality studies and concerns on the unintended consequences of IPA, should be the motivation to continue research. Although some data are available for the penicillins, additional studies including patients with uterine contractions as representatives for IPA-candidates are needed to clarify efficacy. For erythromycin, clindamycin and vancomycin maternal pharmacokinetics and transplacental transfer need to be further investigated in this special patient group. The increase in IPA due to change in strategy to the screening-based approach, adds to the general increase in antibiotic use. Widespread use of antibiotics generally contributes to the increase in resistance. As an alternative preventive strategy interference with the neonatal immune system has been mentioned. Especially the development of a universal maternal vaccine may benefit from the application of genomic/proteomic technologies[114]. However, implication of the current prevention strategy[2] may interfere with clinical vaccine efficacy trials[114]. Furthermore, research on virulence factors within the different GBS types may lead to early detection of virulent GBS strains and thereby narrow the use of IPA to carriers of virulent GBS strains in the future.

Reviewing the evidence for efficacy and unintended consequences, the use of IPA should be limited to patients at risk for GBS-EOD. The unintended consequences of IPA indicate that administration of IPA to all GBS carrying patients is not desirable. Until new information becomes available, the dosing regimen should be continued as recommended by the CDC[2]. Benzylpenicillin is still the antibiotic of first choice. Firstly, because most data are available for this antibiotic, suggesting efficacy. And secondly because the risk on neonatal unintended consequences is limited. Skin testing should be performed in patients suspect for penicillin allergy in history. Clindamycin, and not erythromycin is the

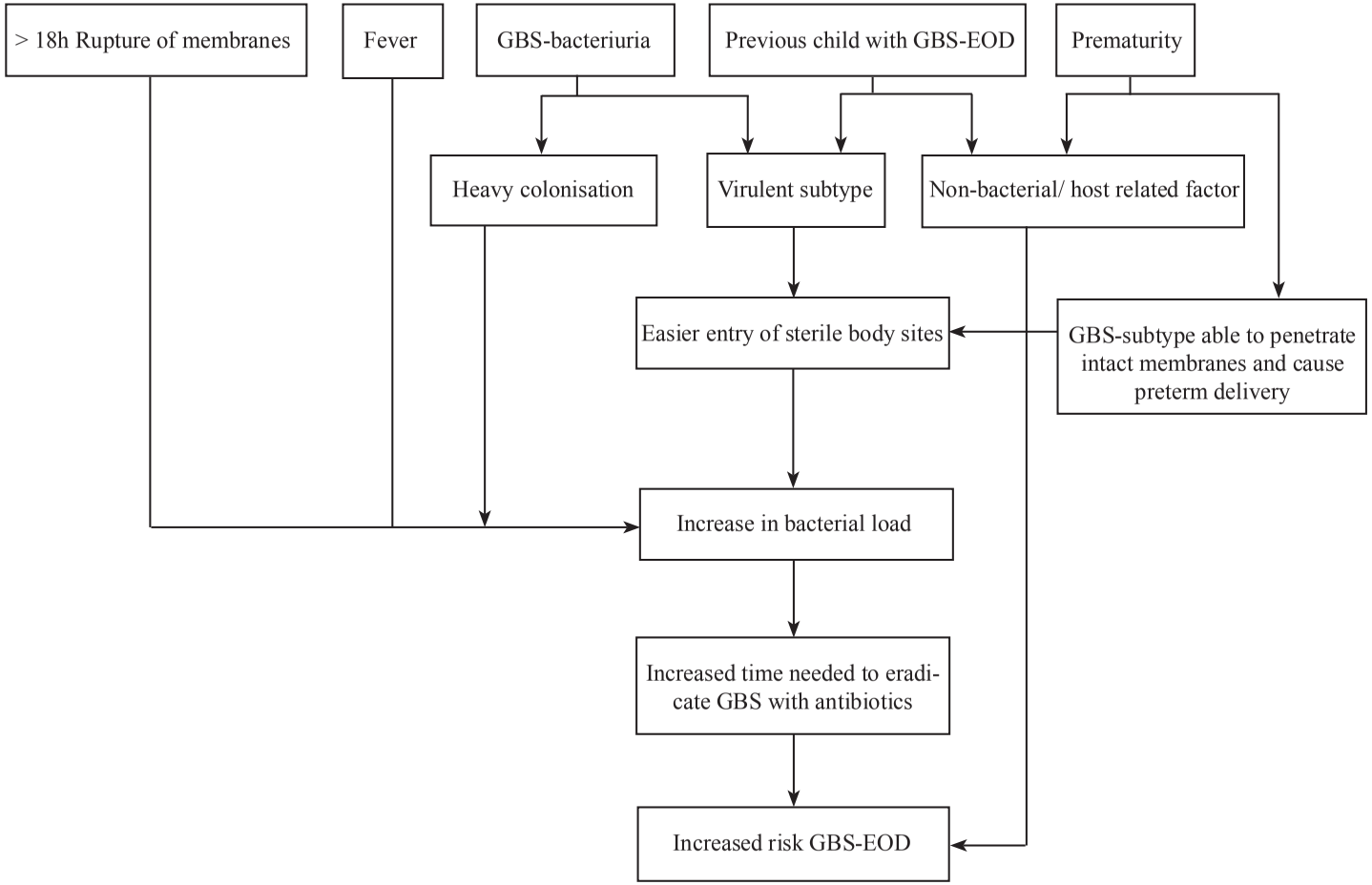


***Figure 3**: Hypothetic interrelationship between risk factors to increased risk of GBS-EOD.*

alternative for penicillin allergic patients. In patients with a negative skin test the administration of benzylpenicillin is recommended. Since fever during delivery is the most important factor for development of GBS-EOD after IPA, neonates from mothers with intrapartum fever should always be admitted to the neonatal care unit.

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Chapter 3

Morbidity related to maternal group B streptococcal infections.

Anouk E. Muller, Paul M. Oostvogel, Eric A.P. Steegers, P. Joep Dörr

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Abstract

Group B streptococcus is known to be a leading cause of neonatal infection, but less appreciated is the fact that it causes maternal infection also. Maternal group B streptococcal infections during pregnancy and delivery threaten not only the mother, but the child as well. Postpartum infection, such as mastitis, bacteremia, sepsis, meningitis, endometritis and wound infections are hazards to the mother. We describe the various maternal group B streptococcal infections, their characteristics, associated neonatal morbidity, and prevention and treatment strategies during pregnancy, delivery and in the postpartum period.

Introduction

Group B streptococcus (GBS, *Streptococcus agalactiae*) has been known as a human pathogen since 1938[1]. It emerged as leading infectious cause of neonatal morbidity and mortality in the 1970s[2]. Because of this, much attention has been given to the prevention of neonatal GBS disease. Guidelines to prevent neonatal GBS disease were developed in the 1990s. After the implementation of these preventive guidelines, the incidence of early onset disease decreased markedly from an estimated 1.8 cases per 1000 live births in 1990 to 0.32 cases per 1000 live births in 2003 in the Unites States[3,4]. Other countries showed a similar decrease. However, despite the decrease in the incidence, GBS remains the number one cause of infectious neonatal morbidity and mortality in the Western world. The majority of cases of early-onset GBS disease occur in infants whose mothers screened negative for GBS colonization[5].

GBS has also been recognized as an important maternal pathogen. A variety of maternal GBS infections may occur in the course of pregnancy and the postpartum period. Apart from cervicovaginal colonization, which is usually asymptomatic, GBS can cause urinary tract infections, vulvovaginitis, intra-amniotic infection, mastitis, bacteremia, sepsis, meningitis, endometritis and wound infections[6].

Because of the serious complications that may affect both mother and fetus, these maternal infections require special attention and proper treatment. In this paper the various maternal infections, their characteristics and the specific prevention and/or treatment strategies are reviewed. The infections are described in the chronological order in which they may be encountered during and after pregnancy.

The pathogen

GBS is a Gram-positive coccus, growing in chains or as diplococci. Because GBS causes complete destruction of red blood cells on sheep blood agar, colonies produce a characteristic appearance with narrow surrounding zones of β-hemolysis. Serologic identification of GBS suspected colonies is performed using latex agglutination.

GBS is serologically classified into nine serotypes based on antigenic capsular carbohydrates as Ia, Ib and II-VIII. Surface proteins are expressed nearly independent of those serotypes. Differences in the expression of carbohydrates and surface proteins account for differences in the pathogenesis of infections[7,8]. Factors playing a role in the development of an asymptomatic or invasive infection have not yet fully been elucidated[7]. As colonization can often occur without symptoms, it is possible that only certain virulent GBS serotypes or surface proteins may cause symptomatic infections. This is currently an area of research[7,8]. However, the present knowledge on the different GBS types is insufficient to be clinically relevant.

During pregnancy

Urinary tract infections

General

Urinary tract infections (UTI) are the most common bacterial infections during pregnancy. GBS causes asymptomatic bacteriuria, cystitis, and pyelonephritis acquired by an ascending route from the vagina. UTI due to GBS are clinically indistinguishable from UTI due to other bacteria in pregnant and in non-pregnant women. GBS bacteriuria, often with low bacterial count, complicates up to 7% of pregnancies[9-11], of which 70% are asymptomatic[12]. The frequency of symptomatic UTI in pregnancy could reflect asymptomatic bacteriuria acquired earlier in life. GBS causes about 10% of the cases of acute pyelonephritis, mainly in the second trimester[13]. Serotype III and non-typable strains are responsible for the majority of bacteriuria[14,15].

Sequelae

UTI due to GBS have been associated with adverse pregnancy outcomes such as (preterm) premature rupture of the membranes ((P)PROM), preterm labor, and neonatal GBS infections, even with low bacterial counts ($<10^2$ bacteria per ml of urine)[12]. Nevertheless, the causal relation between GBS bacteriuria and preterm delivery and PROM is controversial, as several studies report contradictory results [12,16-20].

It has been suggested that GBS bacteriuria may be associated with neonatal GBS disease as well and is therefore one of the commonly used risk factors for neonatal GBS disease[21]. However, there is little evidence for a causal relation. The association with increased neonatal GBS disease is based on two studies, with 10 and 14 patients respectively[9,14]. A third study reported several cases of neonatal GBS sepsis in patients with both GBS bacteriuria and premature delivery[12]. It has been assumed that asymptomatic GBS bacteriuria during pregnancy is associated with heavy genital colonization with GBS[9,10,14], based on an enhanced prevalence of adverse neonatal outcomes. However, no quantitative cultures have been performed in these studies to confirm this. Two studies investigating the relation between bacteriuria and genital colonization reported a positive predictive value of GBS bacteriuria in first trimester of pregnancy for positive GBS genital culture at the time of labor of 30.2%[11] and 61%[22] respectively. McKenna et al. reported that in women with GBS bacteriuria, in only 63% the same serotype was found in the urine and genital cultures[11].

Acute pyelonephritis is a serious threat to maternal and fetal well-being[23]. It can lead to perinatal complications including premature delivery, low birth weight and fetal mortality[15]. Maternal acute pyelonephritis is associated with

anemia, thrombocytopenia, septicemia, transient renal dysfunction, preeclampsia, pregnancy-induced hypertension, and pulmonary insufficiency[13,15].

Prevention

Strong evidence for a causal relation between GBS bacteriuria and adverse outcomes is absent, however screening for bacteriuria early in pregnancy may be considered. GBS bacteriuria should be treated, whether symptomatic or not[24]. Treatment in the first trimester has been shown to reduce the incidence of symptomatic cystitis and pyelonephritis[25]. Treatment of asymptomatic GBS bacteriuria at 28 weeks gestation has been shown to reduce the risk of preterm labor and PPROM in one randomized controlled trial[20].

Though there is evidence for the benefit of treatment of GBS bacteriuria in third trimester as mentioned before, there is no evidence that treatment of GBS bacteriuria in first trimester prevents adverse neonatal outcome. First trimester bacteriuria does not automatically equate to heavy genital tract colonization at 35-37 weeks gestation[11]. Nevertheless, current Centers for Disease Control and Prevention (CDC) guidelines call for intrapartum prophylaxis for early onset neonatal GBS disease if bacteriuria was diagnosed during the pregnancy[21], and thus there is no need for rectovaginal culture at 35-37 weeks in women in whom GBS bacteriuria was diagnosed.

Treatment

There are many antibiotics available for the treatment of urinary tract infections. However, there are insufficient data to recommend any specific regimen in general[26], but during pregnancy the use of nitrofurantoin covers most common microorganisms, such as *E. coli* and other Gram-negative bacteria[26,27].

First choice therapy of (a)symptomatic bacteriuria due to GBS is oral administration of penicillin for 4-7 days[20,28]. The effectiveness of shorter treatment has not yet been proven[28]. One week after completion of the antibiotic treatment the urine-culture should be repeated to confirm the effectiveness. Because of the high recurrence rate of bacteriuria during pregnancy[29], urine cultures should be repeated monthly. GBS pyelonephritis is treated with penicillin G for a total duration of 14 days, starting with intravenous administration. After clinical response to intravenous therapy, treatment should be continued orally. In some settings daily suppressive antibiotic therapy is continued until delivery.

Vaginitis and GBS

General

GBS is a commensal endogenous bacterium in the gastrointestinal tract, which is the likely source of subsequent vaginal colonization. Studies indicate that 10-30% of all pregnant women are colonized with GBS in the gastrointestinal or genital tract[30,31]. Colonization can be transient, chronic or intermittent. Most carriers are asymptomatic.

Bacterial vaginosis associated with various anaerobic bacteria, *Gardnerella vaginalis* and *Mycoplasma hominis*, is the most common cause of vaginitis. It is not clear whether there is an etiological role of GBS in pregnant women for excessive vaginal discharge and symptomatic vaginitis. In non-pregnant women there is some evidence that GBS could be capable of causing symptomatic vaginitis. GBS seems to be more prevalent in patients with purulent or excessive vaginal discharge[32,33]. Vaginitis seems to be related to colonization with GBS[33]. However, there is no consensus whether GBS is the actual causative microorganism in these cases or whether GBS is present only as a cofactor[34,35].

Sequelae

Whether vaginal GBS colonization is a risk factor for PROM, PPROM and preterm delivery is still controversial. Associations between the colonization with GBS and PROM or with preterm delivery have not been found consistently[36-38]. PROM and preterm delivery are risk factors of early-onset neonatal GBS infection.

Vaginal GBS colonization increases the risk on several maternal infections, such as urinary tract infection, endometritis and wound infection. Other additional risks are secondary to PROM, PPROM, and the use of corticosteroids, antibiotics or tocolytic agents.

Studies on GBS transmission in colonized mothers during delivery report incidences between 16-53%[39-42] and neonatal disease develops with a frequency of 1%[40] to 22%[39] in colonized neonates. Only 1-2% of infants of colonized women develop early-onset GBS disease in the first week of life.

Prevention

During labor there are different strategies to prevent neonatal GBS disease. Guidelines from CDC and Canadian guidelines[21,43] recommend universal screening for rectovaginal GBS colonization in pregnant women at 35-37 weeks of gestation and administration of prophylactic antibiotics during labor to all GBS positive women. Prior to these guidelines a risk-based strategy was common in the USA, indicating that only women with a risk factor should receive antibiotics during labor. Risk factors for neonatal GBS disease are prematurity, ruptured membranes for more than 18 hours, fever, GBS bacteriuria in current pregnancy, or a previous

neonate with GBS disease. A risk-based strategy is still being applied in the Netherlands. Another possibility is to combine the risk-based and the screening based strategy and treat only GBS positive women with a risk factor[44]. Schrag et al.[45] demonstrated that routine screening for group B streptococcus prevents more cases of early-onset disease than the risk-based approach.

Treatment
There is no evidence that screening and treatment of asymptomatic bacterial vaginosis reduces adverse neonatal outcome[46]. Symptomatic bacterial vaginosis should be treated with a regimen based on the culture results[46] or empiric with metronidazole[47]. It is advised not to treat GBS colonization before the onset of labor, because recolonization is likely to occur[34,35].

During delivery

Intra-amniotic infection

General
The term intra-amniotic infection (IAI) refers to the clinical syndrome of infection of the placenta and membranes accompanied by signs and symptoms in the mother and/or the fetus. Although the diagnosis is made using clinical symptoms, no universally accepted criteria have been described so far. Commonly used criteria include maternal temperature of >38˚ C, fetal tachycardia (>160 beats/minute), uterine tenderness and foul smelling amniotic fluid[48]. IAI due to GBS occurs after ascending spread from the vagina. The reported incidence varies with the duration of gestation and the criteria used to diagnose IAI. The incidence of IAI based on clinical diagnosis is approximately 1-2% of all term deliveries, but in preterm deliveries the incidence is increased[49]. Bacteria normally present in the vagina are the most common amniotic fluid isolates in women with IAI. GBS was found in 15.4% of the amniotic fluid of patients with IAI and one of the most frequently isolated species in infected newborns delivered of mothers with IAI[50-52]. Colonization with GBS increases the risk on IAI during labor[53]. The risk for IAI increases with the duration of rupture of the membranes. However GBS can sometimes be cultured in amniotic fluid samples from patients with intact membranes as well[54,55].

Sequelae
The risks for maternal and neonatal morbidity and mortality is increased in patients with IAI. Maternal consequences include infection (serious maternal pelvic infections as well as sepsis), prolonged duration of labor, the need for higher doses of oxytocin when uterine stimulation is required[56] and an increased risk for delivery

by caesarean section[57]. Postpartum hemorrhage is more common in these patients, due to impaired myometrial contraction. Bacteremia occurs in 2-6% of patients with IAI. However, when GBS is the cause of IAI much higher incidences of bacteremia are reported (up to in 18%)[58].

Fetal aspiration of infected amniotic fluid can lead to stillbirth, neonatal pneumonia, or sepsis[21]. Neurodevelopmental delay and cerebral palsy are potential long-term disabilities resulting from IAI[59,60].

Prevention

Most neonatal infections are acquired in utero, often without clinical signs of infection. The current CDC guidelines[21] recommend the administration of antibiotics to all GBS positive women during labor to prevent IAI due to GBS. The previously used risk-based strategy advised antibiotics only in situations with an enhanced risk for neonatal GBS disease[61].

The presence of GBS influences the choice of management in patients with PPROM, since subclinical GBS intrauterine infection has been implicated as a major factor in the pathogenesis and consequential maternal and neonatal morbidity. For patients with PPROM and a positive or unknown GBS culture antibiotic therapy is recommended to prevent or treat ascending intrauterine infection[21]. Due to the administration of antibiotics pregnancy will be prolonged and both maternal and neonatal infectious morbidity is decreased[62,63]. Unfortunately, the majority of cases of IAI in the setting of PPROM do not produce the signs and symptoms traditionally used as diagnostic criteria for clinical chorioamnionitis.

Treatment

When signs of infection are present antibiotic treatment is advised and delivery is expedited. Several studies have demonstrated the benefit of intrapartum therapy compared to maternal therapy starting postpartum for both the incidence of neonatal sepsis and maternal morbidity[50,64]. This was especially prominent with sepsis due to GBS. A Cochrane systematic review[65] concluded that the outcome after intrapartum and postpartum treatment was not significantly different probably because the number of patients included was too low[50]. However, it should be noted that the interim analysis of Gibbs' study[50] was in strong favor of intrapartum treatment. Therefore, this study had to be stopped due to clearly worse neonatal outcome in the postpartum treatment group.

It is still under debate what is the optimal treatment regimen should be[65]. Most IAI are caused by either *E. coli* or GBS. However, culture results are not available at the time treatment starts. Therefore, treatment is usually initiated on an empirical basis with a combination of a penicillin for GBS and gentamicin for *E. coli*[50], starting intrapartum. Some authors advise the addition of clindamycin to cover anaerobic bacteria[66,67].

Postpartum infections

Mastitis

General
Mastitis is a parenchymatous infection of the mammary glands, most commonly caused by *Staphylococcus aureus*. Puerperal mastitis due to GBS can be either symptomatic or asymptomatic. The incidence of acute puerperal mastitis varies from 2.9% to 24%. Only one study has examined the breast GBS carriage rate in humans, finding an incidence of 3.5% in 1132 milk samples from healthy lactating mothers[68].

Sequelae
Maternal milk (in cases of either clinical or sub-clinical mastitis) is a potential source of infection resulting in either late-onset (i.e. from one week of life to three months) or recurrent neonatal GBS disease[69-75]. However, the pathogenesis of mastitis is unclear. Most likely, infection of the maternal breast follows colonization of the neonate in the oropharynx acquired during delivery. Afterwards, neonatal infection is thought to occur as a result of aspiration of organisms in the mammary ducts when negative pressure is created by sucking[70]. But it is also possible that GBS has entered the mammary glands prior to labor and does not originate from the neonate itself.

Prevention
It is important to be aware of the possibility of GBS mastitis and GBS carriage in breast milk. Since the pathogenesis of GBS mastitis is unclear, prevention is difficult. In case of suspected or recurrent GBS neonatal disease, breast milk should be cultured and breast feeding stopped until the cultures are negative.

Treatment
Treatment of mild cases of mastitis is conservative, with the use of compresses, rest, and antipyretics. Antibiotics are used only in febrile patients[76]. Empiric therapy consists of cloxacillin, or erythromycin[77]. All cases of GBS mastitis should be treated with ampicillin, because of the possibility of serious neonatal morbidity.

Bacteremia and sepsis

General
Bacteremia in pregnancy and the puerperium may result from common medical illnesses (e.g. pneumonia, appendicitis) or conditions unique to pregnancy (e.g.

endometritis, chorioamnionitis). Among cases of GBS puerperal infection, bacteremia occurred in 31% to 35%[78]. In general, bacteremia progresses to sepsis in 5-25%, while septic shock is rare[79]. Bacteremia within 15 minutes after manual removal of the placenta prior to the administration of prophylactic antibiotics was found in 14% (13 out of 93 patients) of patients in labor who were delivered by caesarean section. GBS are among of the most commonly isolated microorganisms (38%)[80].

Sequelae
The maternal sequelae due to GBS sepsis do not differ from those related to other bacteria. Sepsis remains an important cause of maternal mortality. In developing countries puerperal sepsis is one of the main factors leading to maternal mortality[81]. Nevertheless, in obstetric patients the incidence of death from sepsis is low, as it is estimated at 0-3%, compared to 10-81% in non-pregnant adults[79]. Another maternal complication of bacteremia is meningitis.

Prevention
Several factors predispose for bacteremia and sepsis. In vaginal delivery, prevention of predisposing factors might reduce the risk for bacteremia. Predisposing factors of bacteremia are early gestational age, low birth weight, internal fetal monitoring, and a positive chorioamnionic membrane culture[80]. Predisposing factors of puerperal sepsis include anemia in pregnancy, prolonged labor (at least 12 hours), frequent vaginal examination during labor (more than 5 times) and premature rupture of membranes[82]. To reduce the risk of bacteremia after caesarean delivery, antibiotics should be administered[80].

Treatment
To prevent the sequelae of bacteremia or sepsis rapid intervention with broad-spectrum antibiotics (beta-lactam with aminoglycoside) is required on an empirical basis[83]. In case culture results show GBS bacteremia the narrow-spectrum antibiotic penicillin is sufficient. Microbiological evaluation should include specimens from blood, urine, wound and endometrium. Antibiotic treatment should continue for 5-7 days.

Meningitis

General
The overall incidence of GBS meningitis is 0.3 cases/ 100.000 population[84]. Postpartum maternal GBS meningitis is rare[85]. In the literature only 10 cases have been described. All cases but one followed a vaginal delivery[86,87]. Only one patient

presented meningitis before delivery whereas one patient was diagnosed 6 months postpartum. The other 8 cases manifested between 14 hours and 6 days postpartum. One patient died. None of the mothers had received antibiotic prophylaxis during delivery. One additional case of GBS meningitis has been reported as a likely complication of obstetric epidural anesthesia[88].

Bacterial meningitis usually develops after hematogenous spread. Bacteria then cross the blood-brain barrier into the subarachnoid space. In an experimental animal model, a high degree of bacteremia had been shown to be a primary determinant for meningeal invasion by GBS[89]. To cause meningitis via the bloodstream, bacteria have to escape the host defenses, multiply and reach the threshold level of bacteremia to invade the meninges. Another possible route of infection is after direct inoculation into the cerebrospinal fluid.

Sequelae

For young adults, outcome is related to the level of consciousness and the presence of seizures at the time treatment is initiated. Potential complications are dementia, seizures, hydrocephalus, cerebral infarction, cerebral venous thrombosis and brain abscesses. Ten percent of patients suffer from hearing deficits after bacterial meningitis. In general, about 30-50% of the survivors sustain neurological sequelae after bacterial meningitis[90].

Prevention

Since the degree of bacteremia in the patient seems to be the primary determinant in the pathogenesis of GBS meningitis[89], prevention of bacteremia should also prevent meningitis. The incidence of bacteremia after cesarean delivery is high, but GBS meningitis occurs predominantly after vaginal delivery. This can probably be explained by the fact that antibiotic prophylaxis in cesarean deliveries lowers the bacterial load.

Treatment

To prevent complications from GBS meningitis treatment should start as soon as the diagnosis is suspected. Lumbar puncture and isolation of GBS is diagnostic. Treatment will start empirically with a 3rd generation cephalosporin[91]. GBS meningitis should be treated with penicillin G or ampicillin for a total period of 2-3 weeks intravenously.

Endometritis

General

Endometritis is a more common complication of cesarean section than of vaginal deliveries (11.4 versus 0.4%)[92]. Endometritis following vaginal delivery develops more frequently in women who had pregnancies associated with adverse fetal outcomes including stillbirth, low birthweight, preterm delivery and serious neonatal morbidity[93,94]. Postpartum endometritis can occur up to 6 weeks following delivery.

Risk factors for the development of endometritis include delivery by caesarean section, instrumental delivery, long duration of labor, internal fetal monitoring, frequent vaginal examinations, preterm labor, premature rupture of membranes, manual removal of the placenta, low socioeconomic status, infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, and colonization with GBS[94,95]. Patients with meconium have a higher risk for endometritis[96], probably because the growth of GBS and *E. coli* is enhanced in meconium stained amniotic fluid[97].

In early postpartum endometritis (i.e. within the first 48 hours), GBS is an important contributor as it is most frequently isolated[98]. High-spiking fever (at least 39˚ C) developing within the first 24 hours after delivery may be associated with very virulent pelvic infection caused by either group A or group B streptococcus[53]. In studies of endometritis, GBS has been identified as the sole pathogen in 2 to 14 percent of cases. It appeared the only pathogen more often after vaginal delivery as compared to caesarean section[99,100].

Sequelae

Maternal morbidity associated with endometritis depends on the type of sequelae. Infection can extend to the peritoneal cavity followed by peritonitis and pelvic abscesses and can even cause sepsis. Septic pelvic thrombophlebitis is a rare complication.

Prevention

Several strategies have been suggested for the prevention of endometritis in general. To reduce the incidence of endometritis antibiotic prophylaxis at the time of cesarean delivery has become a common practice[101]. Fernandez et al.[102] demonstrated that a single dose of amoxicillin and clavulanic acid is beneficial after vaginal deliveries. However, low incidence of endometritis after vaginal delivery and preference for the restrictive use of antibiotics make such practice undesired. There is also some evidence that the intravaginal administration of metronidazole gel reduces the incidence of post cesarean endometritis[103]. The efficacy of chlorhexidine before caesarean delivery and the use of methergine in the postpartum period is controversial[104-108].

Strategies specifically aiming at GBS may be helpful as well. The incidence of GBS endometritis declined after the introduction of the GBS prophylaxis[109]. In a longitudinal study Locksmith et al.[110] compared the infection rates following three consecutive protocols for the prevention of GBS disease. In the selective screening protocol, GBS cultures were obtained from women with PPROM or preterm labor and intrapartum antibiotics were administered to all women with positive culture and a risk factor for neonatal GBS disease. In the risk-based protocol, intrapartum antibiotics were given to all women with unknown colonization status and a risk factor for neonatal GBS disease[111]. Under the universal screening protocol, a culture was performed between the 35-37 week of gestation and intrapartum antibiotic prophylaxis given to all women with a positive GBS culture. Under all three protocols the postpartum endometritis rates were reduced[110]. The best success rate was achieved with universal screening[110].

Treatment

Commonly postpartum endometritis is treated with an empiric regimen against mixed aerobic and anaerobic organisms. The combination of clindamycin with once daily gentamicin is appropriate. Once uncomplicated endometritis has clinically improved with intravenous therapy, oral therapy is not needed[112]. In case GBS is detected the same regimen should be followed to cover the often mixed flora causing postpartum endometritis.

Wound infections

General

Infections in perineal and abdominal wounds after delivery can be caused by GBS. Infections with hemolytic streptococci progress rapidly. Cellulitis, lymphangitis, and bleb formations are typical. Watery exudate from the wound is common. Infection of perineal wounds is relatively uncommon, despite the high prevalence of bacteria present at the site of infection. Owen et al. described episiotomy infections to occur in only 0.05% of all cases[113]. Besides technical procedures, duration of cesarean section over one hour and induction of labor increase the risk of wound infection[114].

Early-onset wound infection is commonly caused by group A streptococcus, presenting with systemic illness. Group B streptococcus may present in a similar fashion[115]. It is not known to what extent GBS contributes to the incidence of wound infections. Abdominal wound infections after caesarean section may be caused by the same microorganisms that can be isolated from the amniotic fluid. At caesarean section after rupture of the membranes for at least 6 hours, GBS can be cultured from the amniotic fluid 8% of the time[116].

Sequelae

Episiotomy dehiscence is most commonly associated with infection. Maternal risks include the extension of the infection, fistula formation and sepsis. Failure to treat these infections exposes patients to the risk of necrotizing fasciitis and bacteremia.

Necrotizing fasciitis is a rare obstetric complication. It involves the superficial fascia, subcutaneous tissue, and, occasionally, deeper tissue layers. It can be fatal and is often rapidly progressive and associated with significant tissue necrosis. Initially it is often unrecognized and later it presents as a fulminating disease with marked high mortality. Prognosis depends on the delay of diagnosis, antimicrobial treatment and wide surgical excision of all necrotic tissue[117]. Necrotizing fasciitis arising from an infected episiotomy due to GBS has been described[118]. Necrotizing fasciitis of an episiotomy may extend to the thighs, buttocks and the abdominal wall. Usually symptoms appear from 3 to 5 days postpartum. Risk factors postpartum for necrotizing fasciitis are diabetes mellitus, obesity, hypertension and drug abuse[119].

Prevention

Puerperal endometritis increases the risk of wound infections[114]. Prevention of endometritis is therefore important for the prevention of wound infection. There is some evidence that GBS prophylaxis is also beneficial in the prevention of wound infections[92]. It is unclear whether this is a direct effect of the antibiotics, or indirectly through a reduction in the incidence of endometritis.

General strategies to prevent wound infection and its extension are straightforward and not specific for GBS. Most important is proper hygiene and proper surgical technique.

Treatment

If the wound infection is mild, antibiotics are not required. If the infection is severe, but does not involve deep tissues, a combination of ampicillin and metronidazole should be prescribed. In case deep tissues are involved or first signs of necrotizing fasciitis appear, a combination of broad spectrum antibiotics (penicillin, gentamicin and metronidazole) and surgical treatment are indicated[77]. Necrotizing fasciitis requires wide surgical debridement. GBS may be involved in most types of wound infections, but no specific approach is required.

Conclusion

GBS not only is an important cause of serious neonatal infection, but also causes a variety of maternal infections. These infections cause less morbidity than neonatal infections, but occur more commonly. Especially during the course of pregnancy and delivery GBS can endanger both the mother and fetus. Mastitis may be a cause of late-onset or recurrent neonatal GBS disease. With early recognition and proper treatment, maternal and neonatal severe morbidity and mortality due to GBS infections are rare.

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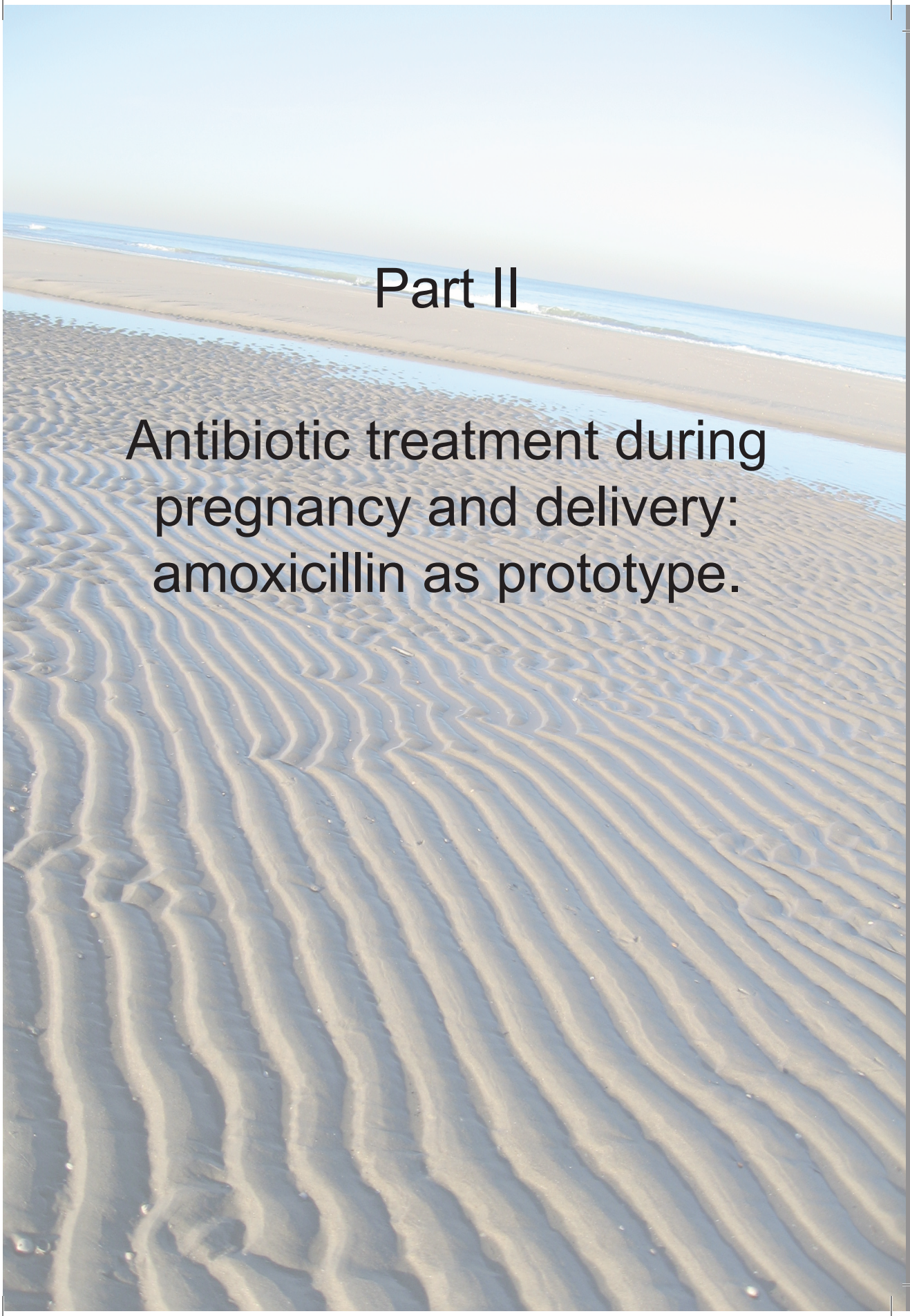

Part II

Antibiotic treatment during pregnancy and delivery: amoxicillin as prototype.

Chapter 4

Amoxicillin pharmacokinetics in pregnant women with preterm premature rupture of the membranes.

Anouk E. Muller, Joost de Jongh, Paul M. Oostvogel, Rob A. Voskuyl, P. Joep Dörr, Meindert Danhof, Johan W. Mouton

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Abstract

Objective: To study the pharmacokinetics of intravenously administered amoxicillin in pregnant women with preterm premature rupture of the membranes (PPROM).
Study design: Healthy women with PPROM were recruited and treated with amoxicillin (2 gram initially and 1 gram subsequently). Blood samples were obtained from the opposite arm and concentrations determined using HPLC. Nonlinear mixed-effects modeling was performed in NONMEM.
Results: The pharmacokinetics of seventeen patients was described by a 3-compartment model. Clearance and volume of distribution at steady state were 22.8 L/h and 21.4 L respectively, similar to values in non-pregnant individuals. There was little variability between patients. No relationship was observed between values of individual pharmacokinetic parameters and various covariates.
Conclusion: The pharmacokinetics of amoxicillin in pregnant patients with PPROM is similar to non-pregnant individuals. Given the small inter-individual variability in pharmacokinetics, no dose adjustments are required to account for differences between subjects under normal circumstances.

Introduction

Preterm premature rupture of the membranes (PPROM) complicates approximately 3% of pregnancies and is responsible for one third of all preterm births[1]. Subclinical intra-amniotic infection has been implicated as a major etiological factor in the pathogenesis of PPROM[2]. The consequential maternal and neonatal morbidity are attributed to ascending infections from the vagina after rupture of the membranes. Antibiotic therapy has been recommended in the management of patients with PPROM to prevent or treat ascending intra-amniotic infection[3,4].

Amoxicillin, a penicillin derivative, is an antibiotic frequently used in the management of PPROM. It is active against common pathogens that can cause infection in neonates, in particular *Streptococcus agalactiae*. The currently recommended amoxicillin dosages in pregnancy are derived from studies using ampicillin[5,6]. These dosage regimens essentially do not differ from regimens employed in non-pregnant individuals and are based on the assumption that pharmacokinetics in pregnancy and in young men are similar[5,6]. In non-pregnant individuals a slow elimination phase has been suggested for penicillin G and amoxicillin[7,8]. Especially for bacteria with a low MIC, like *Streptococcus agalactiae*, a slow elimination phase would be of clinical importance. In women with PPROM the presence of such elimination phase would be beneficial for efficacy of the prophylaxis by increasing the time the amoxicillin concentration remains above the MIC. However, during pregnancy physiological changes occur that may modify the pharmacokinetics of drugs, such as increase in plasma volume, increase in fat content, presence of the fetus, changes in elimination rate or metabolism[9]. These changes can be expected to affect the pharmacokinetics of drugs in various ways. If changes in pharmacokinetics indeed occur, pregnant women and their fetus are inherently at risk for under- or overdosing when they are treated with dosage regimens developed for non-pregnant individuals. A clear example is the drastic decrease in concentration of the antiepileptic drug lamotrigine during pregnancy[10].

Despite the widespread use of amoxicillin in pregnant women, the pharmacokinetics in patients with PPROM has not been adequately studied. The objective of this study is to describe the pharmacokinetics in this vulnerable patient group and to develop a population pharmacokinetic model. Since the pharmacokinetics in individual patients may be affected by various factors, it is important that the method of data analysis allows identification of such factors. Therefore, pharmacokinetic data analysis using Non-Linear Mixed Effects ('population') modeling was applied. This method has distinct statistical advantages, especially for such patient groups. The data of the whole population are simultaneously analysed, while taking into account inter-individual and intra-individual variability in respectively the model parameters and the observations by assuming a stochastic distribution[11]. The influence of specific characteristics on

the individual PK parameters can be assessed by including these characteristics as covariates in the PK-model[12,13]. A more detailed background of population modelling can be found elsewhere[14,15].

Material and methods

Patients

In the period between February 7, 2005 and February 14, 2006, all women with PPROM who needed antibiotic treatment with amoxicillin were eligible for this study. Following the local guidelines, all women with PPROM (gestational age <37 weeks) were admitted to the hospital and monitored for fetal condition and signs of infection. Women with proven or unknown *Streptococcus agalactiae* carriage were treated with antibiotics. Delivery was induced only when signs of infection were present. The study was approved by the Medical Ethics Committee. Written informed consent was obtained from all patients. Women were excluded from the study when i) they had been treated with oral or intramuscular antibiotics within 2 days before starting therapy, ii) were unwilling to comply with the requirements of the study, iii) were known to be allergic to amoxicillin or other penicillins, or iv) were receiving co-medication that exhibits interaction with amoxicillin. All patients were at least 18 years of age and not in labor.

Drug administration and blood sampling

Before the administration of amoxicillin an intravenous catheter was placed in each arm. Amoxicillin was administered following local guidelines. The treatment started with an intravenous infusion of 2 gram amoxicillin (50 mg/mL) administered over 30 minutes, followed by a second infusion after 4 hours of 1 gram amoxicillin over 15 minutes. Bloodsamples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 7 and 15 min (1 gram infusion) or 15 and 30 min (2 gram infusion) during the first two amoxicillin administrations. After the infusion sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30 minutes until the next antibiotic dosage. The exact sampling times were recorded.

Blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 minutes. The supernatants were transferred into plastic storage tubes and frozen at –70° C until analysis.

Patient information

All patients received a standard work-up which included a medical history, biochemical and hematological examination. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded. The amount of edema was scored

semiquantitatively from 0 (no edema) to 3 (above the knee). Before the start of the antibiotics a rectovaginal culture was taken to determine GBS carriage.

Amoxicillin HPLC assay

Amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatography (HPLC) (Shimadzu, Den Bosch, The Netherlands (NL)) method, using an ODS Gemini column (Bester, Amstelveen,NL) with 0.066 M KH_2PO_4 solution containing 10% methanol as a mobile phase. A perchloric acid solution of 0.1 ml was added to the sample in an equal volume and after vortexing, added to 0.56 ml 0.028 M citric acid containing cefadroxil (Sigma, Zwijndrecht,NL) as an internal standard. The assay was linear over the concentration range measured. Controls were included in every run. The lower limit of detection was 0.2 mg/L and the between run CV < 4%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of NonLinear Mixed Effect (population) Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM® software package (version V, release 1.1, GloboMax, Hanover, USA).

To determine the basic structural pharmacokinetic parameters various 1-, 2- and 3-compartment models were tested. Model selection and identification of variability was based on the likelihood ratio test, pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For the likelihood ratio test on differences between two models, the objective function value (OFV) with a pre-specified level of significance of $P<0.001$ was used. NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits the goodness-of-fit plots were visually inspected. The data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero. Population values were estimated for the parameters clearance (CL), the volumes of distribution (V) and the intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log normal distribution. Therefore an exponential distribution model was used to account for inter-individual variability. Possible correlation between inter-individual variability coefficients on parameters was estimated and if present accounted for in the stochastic model (NONMEM Omega block option).

Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots. The residual variability between the observed concentrations and those predicted by the model was described using a proportional error model. The residual error term contains all the error terms which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters were plotted independently against the covariates bodyweight, body mass index, duration of amenorrhea, blood pressure, pulse, oral temperature, and the amount of edema to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance using the likelihood ratio test and the residual intra- and inter-individual variability were visually evaluated. The volume of distribution at steady state (V_{ss}) and terminal half-life ($T_{1/2}$) were calculated following standard procedures[16].

The accuracy of the final population model was established using a bootstrap method in NONMEM, consisting of repeated random sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

Results

In total 17 patients were included. The population consisted of 15 singleton and 2 twin pregnancies. The gestational age at the time of PPROM ranged from 29.4 to 36.9 weeks of pregnancy. The patients were born in 8 different countries, illustrating the heterogeneity of the hospital population as well as the study population. The characteristics of the study patients are presented in table I.

A total of 416 blood samples was collected, which was close to the predefined sampling schedule. The 2 g and 1 g infusions resulted in mean peak concentrations of 96.7 mg/L (range 73.5- 136.6 mg/L) and 70.9 mg/L (n=16, range 49.1-107.1 mg/L), respectively. A three-compartment open model best described the data. The estimates of the pharmacokinetic parameters and their respective coefficients of variation (cv) are summarized in table II. The cv's were relatively small with values between 4.5 and 30.8%. Inter-individual variability was explained by variation in the parameters CL and V_2 (18% for CL and 33% for V_2). This means that the variability between subjects was in fact very small. A correlation between the random parameters for inter-individuality was found and accounted for in the stochastic model. Values of $T_{1/2}$ and V_{ss} were 1.10 h and 21.4 L, respectively.

None of the covariates tested, gestational age, bodyweight, body mass index, blood pressure, pulse, oral temperature, and the amount of edema could

Data	Units	Number of patients	Mean	SD	Range
Maternal age	year	17	29.4	4.64	19.6-35.1
Gestational age	week	17	35.1	1.75	29.4-36.9
Body Mass Index	kg/m^2	17	29.1	3.86	21.5-35
Weight	kg	17	80.6	12.03	56.2-98.9
Edema (no/around the ankle/up to the knee)	-	16	10/5/1		
Leucocytes	$x10^9/L$	17	11.8	4.43	6-25.9
Creatinin	μmol/L	17	44.4	10.11	37-74
Nulliparity	-	11	-	-	-
Twin pregnancy	-	2	-	-	-
Positive maternal GBS culture	-	7	-	-	-

Table 1*: Baseline patients demographic data of the 17 pregnant patients with PPROM. GBS: group B streptococcus (Streptococcus agalactiae)*

improve the model. Finally, no difference in pharmacokinetics between the 2 g and 1g infusion was observed.

The observed and population-predicted profiles for the final model are shown in figure 1. The scatter plot of the observed concentrations versus model-predicted concentrations is shown in figure 2.

The bootstrap validation of the final model was performed with 100 runs. The mean parameter estimates of the runs obtained from the bootstrap analysis did not differ significantly from the predicted values from the NONMEM pharmacokinetic analysis. The standard error obtained from the bootstrap analysis was also comparable to those estimated by the model, except for the intercompartimental clearance between the central and second compartment (Q_1). This value differs significantly from the standard error estimated by the model due to the small size of the study population. The mean values and standard errors are represented in table II.

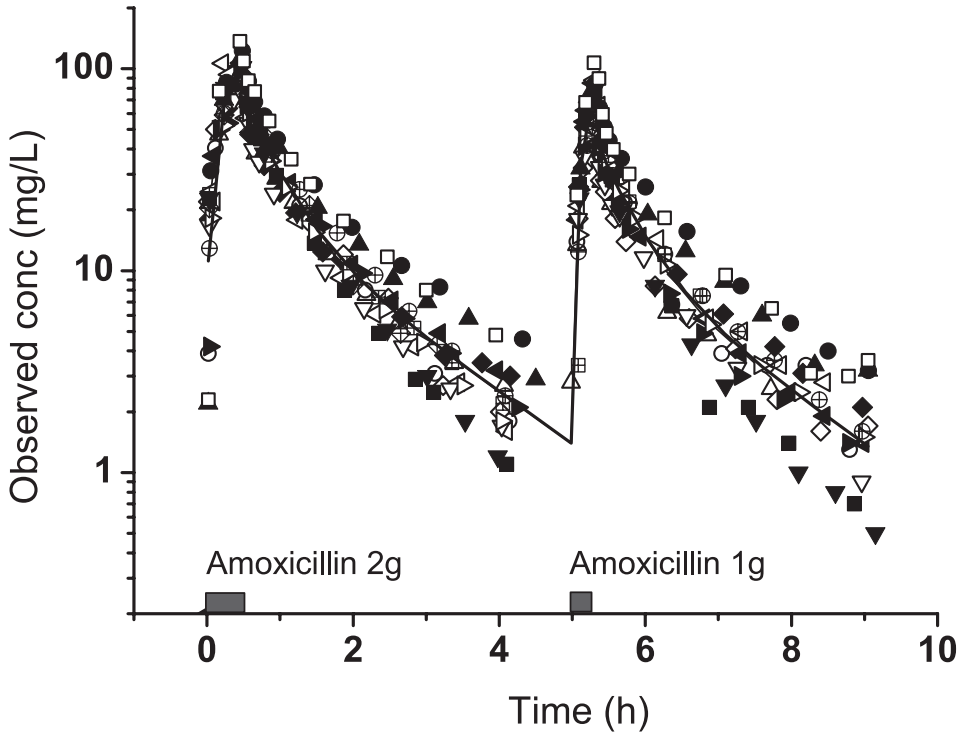

***Figure 1**: Observed concentration-time profiles.*

The superimposed bold line shows the predicted profile obtained with the final model. The blocks indicate the time at which the infusions of the amoxicillin was started and stopped. Because there was variation in the start-time of the second infusion due to the clinical situation, in this graph the data were adapted assuming that the second infusions started at t=5.05h for all patients. (See color inlay for a full color version of this figure.)

Comment

In this study a PK-model was developed to describe the pharmacokinetics of amoxicillin in pregnant women with PPROM. The pharmacokinetics in our population appears to be only slightly different from non-pregnant individuals with a V_{ss} of 21.4 L and a $T_{1/2}$ of 1.10 h. The variability between the patients was small.

parameter	units	Final model estimates		Bootstrap estimates*	
		mean	SE	mean	SE
structural model parameters					
CL	L/h	22.8	1.03	22.8	1.04
V_1	L	5.59	0.826	5.26	1.32
V_2	L	7.43	1.06	7.75	1.16
V_3	L	8.61	0.768	9.07	0.99
Q_1	L/h	60	18.5	82.2	69.8
Q_2	L/h	7.72	1.72	7.92	1.78
variance model parameters					
interpatient variability in CL		0.0317	0.0112	0.0300	0.0106
interpatient variability in V_2		0.108	0.00440	0.0982	0.0476
residual variability		0.0365	0.00492	0.0359	0.00508
derived pharmacokinetics parameters					
$T_{1/2}$	h	1.10	-	-	-
V_{ss}	L	21.4	-	-	-

SE: standard error of the estimate; * mean of 100 bootstrap analyses. The parameter values were compared with the bootstrap estimates using the unpaired t-test.

***Table 2**: Population model parameter values and bootstrap estimates.*

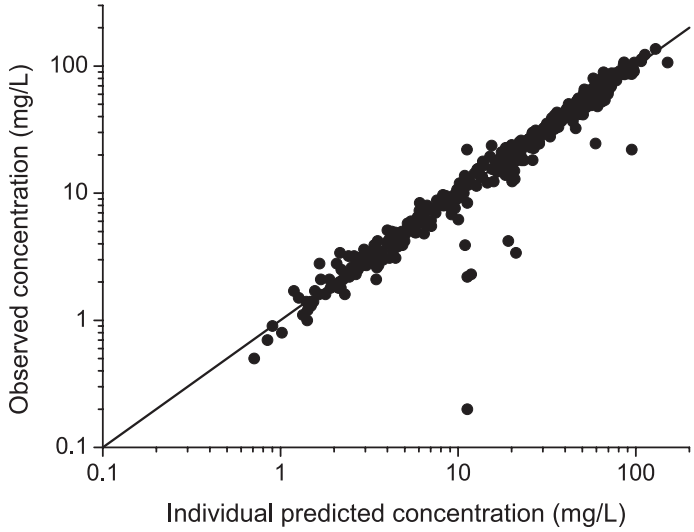

Figure 2*: Scatter plot of the individual predicted versus observed concentrations of amoxicillin for 17 patients. The correlation coefficient was 0.97. The figure shows the individual data points for the entire population and the line of identity (x=y).*

With regard to amoxicillin, values for volumes of distribution, clearance and terminal half-life were all within the ranges reported in the literature for healthy non-pregnant individuals (table III). Only slightly lower peak serum concentrations were observed compared to 7 healthy non-pregnant individuals[17], i.e. 96.7 mg/L and 139.3 mg/L respectively for the 2 gram infusion. The value for V_{ss} in our study was slightly larger than values found by Dalhoff et al. in healthy volunteers[8], who also used a 3-compartment model. Possibly this was due to the increased extracellular fluid in pregnant women and the pregnancy itself.

To our knowledge this is the first study on the pharmacokinetics of amoxicillin in pregnant women. Therefore, direct comparison with other studies under the same conditions is not possible. However, a comparison can be made to studies on ampicillin, which is closely related to amoxicillin (p-hydroxyampicillin). The two compounds differ very little in pharmacokinetics in healthy volunteers, except with respect to absorption after oral administration[18,19]. Several studies have been performed on the pharmacokinetics of ampicillin in pregnancy. In contrast to our study most studies on ampicillin did show differences in the pharmacokinetics during pregnancy, e.g. shorter half-life and higher plasma clearance during pregnancy[20-24]. This dissimilarity is intriguing; possible explanations are the use of different methods of analysis or the inclusion of patients at different gestational age and circumstances.

Two studies have noted that the clearance of amoxicillin after an intravenous dose exhibited a statistically significant dose effect[17,25]. However, the 2 studies are inconsistent with respect to the range where deviation of linearity occurs. Mastrandrea et al. described a difference in clearance in the range from 500 mg to 1000 mg[25], whereas Hill et al. found a slight deviation from linearity after a 5 g dose compared with doses of 250-1000 mg[17]. In a study by Sjövall et al. the pharmacokinetics after infusions in doses ranging from 1.9 g and 2.8 g were linear[19]. In our data, covering the range of 1-2 g, there was no evidence for a dose effect on the clearance. It is unlikely that therapeutic consequences are to be expected.

In general, inter-individual variability in pharmacokinetic parameters observed in clinical study populations are due to biochemical and physiological differences between subjects. In association with pregnancy, additional physiological alterations occur, which may further increase the variation in parameters between individuals in pregnant populations[26]. Surprisingly, the inter-individual variation in our data was remarkably small. While this was an unexpected finding, from the clinical perspective this is convenient, because specific adjustments are unnecessary for this patient group.

An important question is whether this dosing regimen is adequate to treat or prevent morbidity in both mother and fetus. The efficacy of the penicillins is determined by the time the concentration exceeds the minimum inhibitory concentration (T>MIC) and, in general, T>MIC for 40-50% of the dosing-interval is required for efficacy[27-29]. The breakpoint MIC value of an antibiotic used is the highest MIC value of different causative microorganisms that results in a high probability of cure, as follows from the target T>MIC. Since rectovaginal carriage of *Streptococcus agalactiae* has been described in up to 30% of pregnant women, this is an important microorganism after PPROM in the development of neonatal infection[3,30]. MIC values of amoxicillin for *Streptococcus agalactiae* are scarce, but vary from 0.03 to 0.12 mg/L[31,32]. The peak serum concentrations in our pregnant population were slightly lower than in non-pregnant individuals, but nevertheless well above the MIC. More importantly, maternal serum concentrations remained above the MIC for sufficient percentage of the dosing interval (>95%), even taking into account the protein binding of amoxicillin. The presence of a slow elimination phase, represented by the third compartment, significantly contributes to the high value for T>MIC. Because amoxicillin reaches the fetus after transplacental transport, it should be noted that adequate maternal levels are a prerequisite for the prevention of fetal infection, but no guarantee. In treatment of the mother, the added value of a 2 g loading dose above a 1 g dose is doubtful. However, it remains to be confirmed that by using this dosing schedule adequate fetal and AF levels are established as well.

Author	Dose (mg)	Infusion Time (min)	V_1 (L/kg)	V_2 (L/kg)	V_3 (L/kg)	V_{ss} (L/kg)	CL (L kg^{-1} h^{-1})	$T_{1/2}$ (h)	Number of Patients (F/M)
Zarowny[33]	250	33	0.187	0.095	-	0.282	0.264	1.05	0/8
Spijker[34]	250	0.17	0.17	0.12	-	0.29	0.32		0/8
Spijker[34]	500	0.17	0.22	0.24	-	0.46	0.32		0/8
Spijker[34]	1000	0.17	0.22	0.27	-	0.49	0.34		0/8
Dalhoff[8] 1*	250/500/1000	0.5	0.093	0.100	-	0.193			0/7
Dalhoff[8] 1*	2000/5000	30	0.129	0.084	-	0.213			0/7
Dalhoff[8] 2*	250/500/1000	0.5	0.075	0.06	0.069	0.205		1.63	0/7
Dalhoff[8] 2*	2000/5000	30	0.119	0.056	0.069	0.246		3.32	0/7
Adam[35]	4000	5	0.077	0.065	-	0.14	-	1.11	6/6
Arancibia[36]	500	0.17	0.18	0.070	-	0.25	0.20	1.08	2/7
Mastrandrea[25]	500	bolus					14.8 (L/h)	1.16	0/20
Mastrandrea[25]	1000	bolus					20.7 (L/h)	1.26	0/20
Hill[24]	250/500/1000	0.5						1.18	0/7
Hill[24]	2000/5000	30						1.26	0/7
This study	**1000/ 2000**	**15/30**	**0.0694**	**0.0923**	**0.107**	**0.268**	**0.283**	**1.10**	**17/0**

* Dalhoff et al. analyzed the same concentration-time data using a two-compartment model (1) and a three-compartment model (2). V_1 Volume of distribution of central compartment; V_2 Volume of distribution of first peripheral compartment; V_3 Volume of distribution of second peripheral compartment; V_{ss} Volume of distribution at steady state; CL clearance; $T_{1/2}$ terminal half-life; F female, M male

***Table 3**: Pharmacokinetic parameters of amoxicillin in healthy (non-pregnant) volunteers reported in the literature and the results from our study.*

It is surprising that the pharmacokinetics in pregnant women with PPROM did not differ significantly from non-pregnant individuals. However, it should be noted that this is only valid for pregnant women with PPROM who are otherwise healthy. It has been suggested previously that it is not the state of pregnancy that influences the pharmacokinetics, but being in labor[6]. Since our patients were not in labor, this might explain why our data were similar compared to previously reported data of non-pregnant individuals.

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Chapter 5

The influence of labor on the pharmacokinetics of intravenously administered amoxicillin in pregnant women.

Anouk E. Muller, P. Joep Dörr, Johan W. Mouton, Joost DeJongh,
Paul M. Oostvogel, Eric A.P. Steegers, Rob A. Voskuyl, Meindert Danhof

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Abstract

Aim: Many physiological changes take place during pregnancy and labor. These might change the pharmacokinetics of amoxicillin, necessitating adjustment of the dose for prevention of neonatal infections. We investigated the influence of labor on the pharmacokinetics of amoxicillin.

Methods: Pregnant women before and during labor were recruited and treated with amoxicillin intravenously. A postpartum dose was offered. Blood samples were obtained and amoxicillin concentrations were determined using high-pressure liquid chromatography. The pharmacokinetics was characterized by nonlinear mixed-effects modeling using NONMEM.

Results: The pharmacokinetics of amoxicillin in 34 patients was best described by a 3-compartment model. Moderate inter-individual variability was identified in CL, central and peripheral volumes of distribution. The volume of distribution increased with an increasing amount of edema. Labor influenced the parameter estimate of peripheral volume of distribution (V_2). V_2 was decreased during labor, and even more in the immediate postpartum period. For all patients the population estimates (mean +/- SE) for CL and the volumes of distribution (V) were 21.1+/-4.1 L/h (CL), 8.7+/-6.6 L (V_1), 11.8+/-7.7 L (V_2) and 20.5+/-15.4 L (V_3) respectively.

*Conclusion:*The peripheral distribution volume of amoxicillin in pregnant women during labor and immediately postpartum is decreased. However, these changes are not clinically relevant and do not warrant deviations from the recommended dosing regimen for amoxicillin during labor in healthy pregnant patients.

Introduction

The use of antibiotics during labor for prevention of neonatal infections has increased substantially in the last decades. Current guidelines from the Centers of Disease Control and Prevention (CDC) to prevent neonatal group B streptococcal (GBS) disease recommend the use of antibiotics during labor in all pregnant women carrying GBS[1]. The prevalence of GBS carriage varies according to the geographical region from 10% to 35% of all pregnant women, resulting in antibiotic use for this purpose in up to one of every three women[2-5]. Amoxicillin, a penicillin derivative, is one of the antibiotics used in the prevention of GBS disease. Amoxicillin is widely used in Europe, while ampicillin is more commonly used in the US. Amoxicillin is a bactericidal antibiotic and is primarily excreted unchanged in the urine both by glomerular filtration and by tubular secretion in the kidneys.

Adequate dosing of antibiotics to the mother is essential to prevent the neonate from GBS disease. Antibiotics reach the fetus after transplacental passage. An adequate concentration-time profile in maternal serum is therefore a prerequisite for an adequate concentration-time profile in the fetus. Current dosing regimens are similar for non-pregnant individuals, pregnant women before the onset of labor and for pregnant women during labor. Many physiological changes take place during pregnancy, which may modify the concentration-time profile of specific drugs, such as an increase in plasma volume, presence of the fetus and changes in elimination rate or metabolism[6]. In a previous study we found no differences in the pharmacokinetics (PK) of amoxicillin between pregnant women with preterm premature rupture of the membranes (PPROM) and values reported in the literature for non-pregnant individuals[7]. During labor additional physiological changes occur of which many are expected to change the PK behavior of drugs[8]. Uterine contractions, mechanical compression of blood vessels by the gravid uterus as well as hyperventilation, might all have their separate circulatory influences affecting blood flow through the body and especially to the eliminating organs. The consequential change in concentration-time profile of the antibiotic for both mother and neonate are unpredictable.

Despite the regular use of amoxicillin during labor, the influence of labor on the PK has not been adequately studied. PK studies during labor face considerable ethical and practical difficulties, limiting the collection of blood samples. The number of blood samples collected in women during labor will therefore be smaller compared to pregnant women before the onset of labor. Unbalanced study groups harbor statistical problems. Non-Linear Mixed Effects Modeling (NONMEM)[9] allows weighted analysis of observations from unbalanced study designs and incorporation of patients with small or incomplete datasets[10, 11]. Studying the whole population as one group, the influence of specific circumstances, such as the presence of labor, on the individual PK parameters can be assessed using covariate

analysis[12,13]. A more detailed background of population modeling can be found elsewhere[14,15]. The objective of this study is to investigate the influences of labor on the PK of intravenously administered amoxicillin.

Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women with gestational age of minimally 26 weeks who needed antibiotic treatment with amoxicillin were eligible for the study. From a subset of patients a part of the data was used in a previous study[7]. These patients were all diagnosed with preterm premature rupture of the membranes and therefore before the onset of labor (in total 416 samples). Following the local guidelines, all women with proven or unknown *Streptococcus agalactiae* carriage were treated with antibiotics when pregnancy was complicated by one of the following factors: preterm premature rupture of the membranes, rupture of the membranes for >18 hours, prematurity, fever (>37.8° C), bacteriuria in current pregnancy and a previous child with invasive GBS disease. The choice of the antibiotic for this study was dictated by the local guidelines, which recommend amoxicillin as first choice. Women were admitted to the hospital and monitored for fetal condition, onset of labor and signs of infection. When intra-amniotic infection was suspected, delivery was induced. The study was approved by the Medical Ethics Committee of the Medical Center Haaglanden, the Hague, the Netherlands. Written informed consent was obtained from all patients. Women were excluded from the study when i) they had been treated with oral or intramuscular antibiotics within 2 days before starting therapy, ii) were unwilling to comply with the requirements of the study, iii) were known to be allergic to amoxicillin or other penicillins, or iv) were receiving co-medication that exhibits interaction with amoxicillin. All patients were at least 18 year of age.

Both pregnant patients before the onset of labor and patients in labor were included in the study. Patients included before the onset of labor or during labor, who agreed to receive an additional dose of amoxicillin after their delivery for study purposes only were kept in the study until maximally 28 hours after delivery. Being in labor was defined as the presence of uterine contractions resulting in progressive cervical dilatation. The vaginal examinations were performed by the physician responsible for the delivery. When labor started during the study period, the time of the onset of labor was recorded.

All patients received a standard work-up that included a medical history, biochemical and hematological examination. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded at the onset of the study. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded at the onset of the study. Temperature and pulse were recorded to register the possibility of

intra-amniotic infection, whereas the blood pressure and the amount of edema were recorded to account for differences in distribution volumes. The amount of edema was scored semiquantitatively from 0 (no edema) to 3 (above the knee).

Drug administration and blood sampling

Before the administration of the amoxicillin, an intravenous catheter was placed in each arm. Amoxicillin was administered according to local guidelines in the hospital. Treatment started with an intravenous infusion of 2 gram amoxicillin (50 mg/mL) administered over 30 min, followed by an infusion of 1 gram amoxicillin over 15 minutes every 4 hours until delivery. In the prevention of GBS, antibiotics were administered until delivery. For the purpose of this study only, a single additional dose of 1 gram amoxicillin was administered after delivery 4 hours after the last dose before child birth. Blood samples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 7 and 15 min (1 gram infusion) or 15 and 30 min (2 gram infusion). After the infusion, sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30 minutes until the next antibiotic dosage. Blood samples were collected when possible, taking into consideration the physical and emotional inconvenience to the woman. The exact sampling times were recorded.

Blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 min. The supernatants were transferred into plastic storage tubes and frozen at -70˚ C until analysis.

Amoxicillin HPLC assay

Amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatography (HPLC) (Shimadzu, Den Bosch, The Netherlands (NL)) method, using an ODS Gemini column (Bester, Amstelveen,NL) with 0.066 M KH2PO4 solution containing 10% methanol as a mobile phase. A perchloric acid solution of 0.1 ml was added to the sample in an equal volume and after vortexing, added to 0.56 ml 0.028 M citric acid containing cefadroxil (Sigma, Zwijndrecht, NL) as an internal standard. The assay was linear over the concentration range measured. Controls were included in every run. The lower limit of detection was 0.2 mg/L and the between run CV < 4%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE with INTERACTION method. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston,

Texas, USA) and NONMEM® software package (version VI, release 1.2, ICON Development Solutions, Ellicott City, Maryland, USA).

To determine the basic structural pharmacokinetic parameters various 2- and 3-compartment models were tested. Model selection and identification of variability was based on evaluation of the mean objective function value (OFV), pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For differences between two structural models, the OFV with a pre-specified level of significance of $p<0.001$ was used (corresponding to a difference in OFV of 10 points). NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits, the goodness-of-fit plots were visually inspected. The data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero. Population values were estimated for the parameters clearance (CL), the volumes of distribution (V) and the intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log-normal distribution. Therefore an exponential distribution model was used to account for inter-individual variability. Possible correlation between inter-individual variability coefficients on parameters was estimated and if present accounted for in the stochastic model (NONMEM Omega block option).

Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots. A proportional error model, additive error model and a combined proportional-additive error model were tested to describe the residual variability between the observed concentrations and those predicted by the model. The residual error term contains all the error terms which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters, on which a random effect has been identified, were plotted against the covariates bodyweight, body mass index, gestational age, blood pressure, pulse, oral temperature, and the amount of edema, single or twin pregnancy, creatinin, the renal function calculated with the Cockcroft-Gault (CG) and the modified Modification of Diet in Renal Disease (MDRD) equation to determine whether this influenced the pharmacokinetics[16,17]. Covariate analysis was performed by forward addition of each candidate covariate into the model structure until no further improvement of goodness of fit was observed. A significance level of 0.05 was selected (corresponding to difference in OFV 3.84 points). A further criterion for acceptance of covariate effects was that the estimated 95% confidence interval of the covariate effect did not overlap with zero. Contribution of each covariate to the final model was the confirmed by backward elimination of each covariate from

the model to account for possible interaction between covariates. The residual intra- and inter-individual variability were visually evaluated. The volume of distribution at steady state (V_{ss}) and terminal half-life ($T_{1/2}$) were calculated following standard procedures[18].

Finally, the effect of the presence of labor was investigated on structural PK parameters. The state of being pregnant but before the onset of labor, being in labor and being in the immediate postpartum period were implemented in the model as covariate in the entire group of patients. A significance level of 0.05 was selected (corresponding to difference in OFV of 3.84 points). A further criterion for acceptance of the influence of labor was that the estimated 95% confidence interval of its effect did not overlap with it's null value. Contribution of an effect of labor to the final model was the confirmed by backward deletion of both the effect of labor and the continuous covariates from the model to account for possible interaction between covariates and the effect of labor.

The accuracy of the final population model for the entire population was established using a bootstrap method in NONMEM, consisting of repeated random sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

Results

In total, 34 patients were included. From 8 patients blood samples were taken both before the onset of labor and during labor. From 17 patients blood samples were taken only before the onset of labor and from 9 patients only during labor. Eight patients agreed with a postpartum dose of amoxicillin as well. The postpartum doses of amoxicillin were administered between 1.5-3.8 hours after child birth. The study population consisted of 31 singleton and 3 twin pregnancies. All 17 patients participating in the study during labor, delivered vaginally. The gestational age at the time of the study ranged from 30.0 to 40.6 weeks. The characteristics of the study patients are presented in Table 1.

Peak concentrations were comparable in patients during labor and in patients before the onset of labor. Peak concentrations after the 2 gram infusion were 97.4+/- 20.7 mg/L in patients before the onset of labor and 92.3+/- 16.6 mg/L in patients during labor (mean+/-SD). Peak concentrations after the 1 gram infusion were 71.8+/- 14.8 mg/L, 62.8+/- 7.9 mg/L, and 65.7+/- 15.5 mg/L, respectively, in patients before labor, during labor and postpartum. The terminal half-lives for the three stages of labor were not significantly different (1.1 +/- 0.3 h during labor, 1.2+/- 0.2 h before labor and 1.2+/- 0.2 h postpartum). The volume of distribution in steady state was 40.4 L.

Data	units	Mean	SD	Range	Number of patients
Maternal age	year	29.0	5.5	20-38	34
Gestational age	week	35.9	2.3	30-41	34
Body Mass Index	Kg/m^2	28.8	5.0	18-38	33
Weight	Kg	79.0	13.6	53-107	33
Leucocytes	$x10^9/L$	12.5	5.2	6-28	34
Creatinin	µmol/L	45.1	8.2	30-74	34
Nulliparity	-	-	-	-	15
Edema (no/around the ankle/up to the knee	-	-	-	-	21/10/2

Table 1*: Baseline patients' demographic data of 34 pregnant women.*
SD= Standard Deviation

A total of 898 blood samples were collected in this study. Of these samples 550 were taken before the onset of labor, 187 during labor and 161 in the immediate postpartum period. For patients included before the onset of labor between 7 and 34 samples were obtained per patient, during labor between 5 and 24 samples and in the postpartum period between 12 and 25 samples. A three compartment open model best described the data. The residual error was found to be proportional to the blood concentrations. Inter-individual variability was mainly observed in clearance (CV 19.8%), V_1 (CV 23.1%) and V_2 (CV 31.6%). Correlations between the random parameters for inter-individual variability were found and implemented in the model. For the selected continuous covariates, there was a significant effect of edema on the total volume of distribution. The volume of distribution increased with an increasing amount of edema (Figure 2b). Furthermore, the effect of renal function on CL was found inconsequential. Using the serum creatinin concentration and the estimated creatinin clearance calculated with the CG-formula, no influence on the CL of amoxicillin was seen. However, renal clearance was found to have a small, but significant effect on CL when calculated using the modified MDRD formula. CL was inversely correlated with an increased MDRD (Figure 2C). In figure 1 the observed concentrations are plotted versus the model-based population predicted concentrations, illustrating the unbiased model-fit.

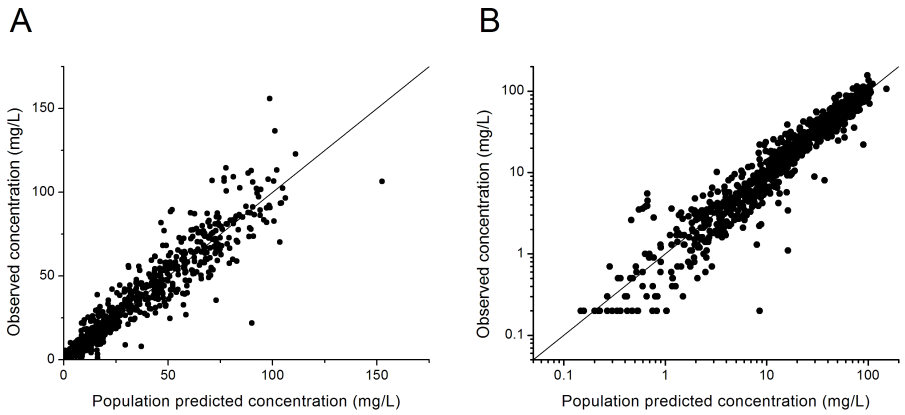

Figure 1
Scatter plot of the population predicted vs observed concentrations of amoxicillin for 34 patients. The figure shows the individual data points for the entire population and the line of identity (x=y) with linear scale (Fig 1A) and logarithmic scale (Fig 1B).

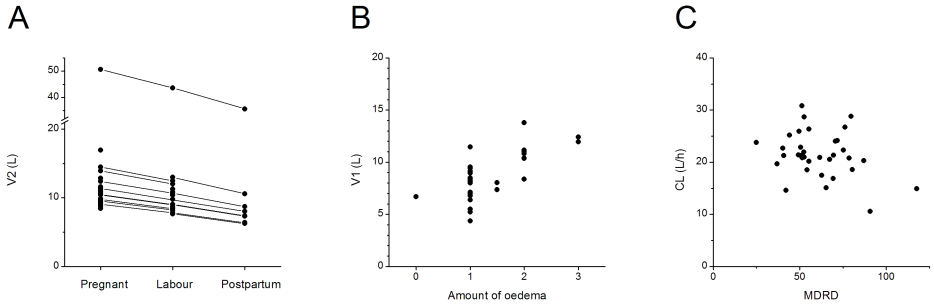

Figure 2
Plots of values for V_2 for the three stages of labor (figure 2a), values of V_1 versus the amount of edema (figure 2b) and values of MDRD versus CL (figure 2c).

Labor status in patients was found to have a small but significant effect on peripheral distribution volume (V_2). V_2 was larger in women before the onset of labor compared to women during labor and in the postpartum period. Compared to women before the onset of labor, V_2 was decreased with 13.7% during labor and 29.5% in the immediate postpartum period. Figure 2a shows the different values for V_2 for the three stages of labor . Figure 3a shows the observed concentrations of patients before and after the onset of labor for the first 4 h after the 2 gram infusion, whereas figure 3b shows the observed concentration for all three stages of labor after a 1 gram dose. The estimates of the pharmacokinetic parameters of the final model and the relative standard errors derived from the bootstrap analysis are presented in Table 2.

The bootstrap validation of the model of the entire population was performed with 100 runs. The mean parameter estimates of the runs obtained from the bootstrap analysis did not differ significantly from the predicted values from the NONMEM PK analysis (data not shown). The bootstrap validation was successful for 95 runs. The standard errors obtained from the bootstrap analysis were also comparable to those estimated by the model.

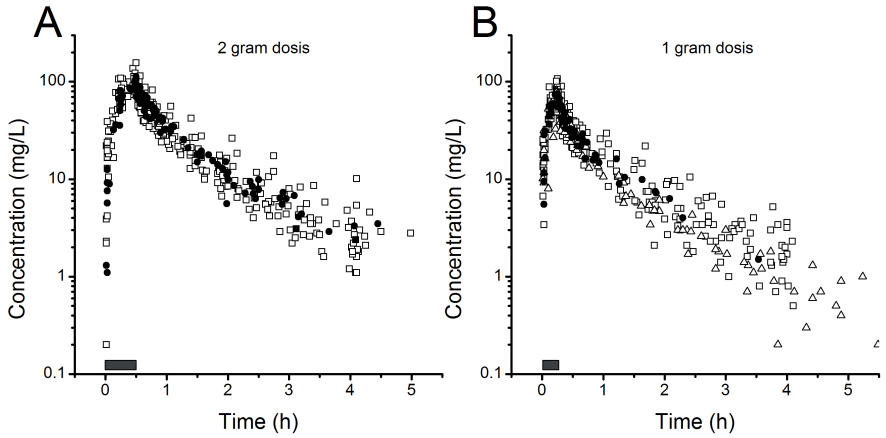

Figure 3

Figure 3a shows the observed amoxicillin concentrations after a 2 gram dose and figure 3b after a 1 gram dose. Time of infusion is indicated by black bars. The open squares represent all data points of the patients before the onset of labor; the filled dots data points of patients during labor and data points of patients in the postpartum period are indicated by the open triangles. Because there was variation in the start-time of the second infusion, in figure 3b the data were adapted assuming that the 1 gram infusions started at t=0 for all patients. (See color inlay for a full color version of this figure.)

Parameter	**Units**	**Estimates of all patients**		
Structural model parameters				
		Mean (model)	**SE% (bootstrap)**	**95%CI (bootstrap)**
CL	L/h	21.1	4.1	19.6 - 23.0
V_1	L	8.7	6.6	7.5 - 9.8
V_2	L	11.8	7.7	9.9 – 13.4
V_3	L	20.5	15.4	14.3 – 26.7
Q_1	L/h	21.9	16.9	15.0 – 29.8
Q_2	L/h	1.5	41.3	0.28 – 2.69
Variance model parameters				
		Mean ($x10^{-2}$) (model)	**CV% (bootstrap)**	**95%CI ($x10^{-2}$) (bootstrap)**
IIV in CL		4.2	19.8	1.6 – 6.1
IIV in V_1		5.1	23.1	-0.16 – 10.6
IIV in V_2		9.7	31.6	1.1 – 17.9
Residual variability		4.6	21.5	3.3 – 5.7

SE: standard error of the estimate, SE%: relative SE (%), 95%CI: 95% confidence interval, CL: clearance, V_1: volume of distribution of the central compartment, V_2: volume of distribution of the first peripheral compartment, V_3: volume of distribution of the second peripheral compartment, Q_1: intercompartmental clearance between V_1 and V_2, Q_2: intercompartmental clearance between V_1 and V_3, IIV: Inter-individual variability, CV%: relative coefficient of variation (%)

Table 2: *Population model parameter values with standard error of 34 women presented with SE% or CV% and 95% confidential interval as derived from the bootstrap analysis.*

Discussion

The PK of intravenously administered amoxicillin of our entire study population was best described by a three-compartment open model. Volume of distribution increased with an increasing amount of edema. An effect of labor was seen on the peripheral volume of distribution (V_2). When compared to pregnant women before the onset of labor, V_2 was decreased during labor and even more decreased in the immediate postpartum period. For our patients dose adjustments were not indicated, but it can not be excluded from this study that the current dosing schedule is adequate for patients with pregnancy-related disorders.

Estimation of glomerular filtration rate (GFR) in pregnant women is difficult. Serum creatinin levels are often used to estimate GFR, especially in conjunction with either the Cockcroft-Gault (CG) or Modification of Diet in Renal Disease (MDRD) formulae[16,17]. Both the CG and MDRD formulae are based on cohort studies of patients with mild to moderate renal insufficiency, and none of the subjects was pregnant[16,17]. Unfortunately, estimation of GFR in the pregnant population using either the CG or the MDRD formula has not been validated. There have been several reports demonstrating that the MDRD formula tends to underestimate GFR in subjects with normal or near-normal renal function[19,20]. Whereas the CG formula is weight based, and weight gain in pregnancy will obviously exaggerate estimated GFR. Alper et al, investigated the GFR in preeclamptic patients. Neither the CG or the MDRD formula were very accurate in predicting GFR in this group of patients, although the MDRD formula performed modestly better[21]. In our analysis, both serum creatinin values and estimated GFR using the CG-formula did not have a significant effect on CL, but when GFR was estimated with the MDRD formula the CL decreased with an increasing GFR. This is unlikely, and therefore illustrates the importance of the use of validates formulas in special patient groups. Pharmacokinetic parameter estimates did not change after the implementation of GFR calculated with the MDRD formula. Based on the absence of an effect of serum creatinin values and the GFR estimated using the CG formula, we concluded that CL is not influenced by renal function.

Other studies on the influence of labor on the PK of amoxicillin are not available. One study has investigated the PK of oral amoxicillin after a single dose both in the second and third trimester of pregnancy as well as postpartum[22]. It was found that during pregnancy the renal clearance was increased compared to the postpartum period[22]. However, in the study of Andrew et al[22] patients were included 3 months after delivery, while our patients were measured within the first 27 hours after delivery. This probably explains the difference between the two studies. The PK of ampicillin, an antibiotic closely related to amoxicillin, has been studied both during pregnancy and during labour[23-28]. Differences in PK of ampicillin between pregnant and non-pregnant individuals have been described, such as a shorter

half-life and higher plasma clearance during pregnancy[25,26,28]. Furthermore, an effect of labor on the terminal half-life of ampicillin has been described[24]. Labor increased the terminal half-life in patients in labor, compared to pregnant women before the onset of labor from 39.2 +/- 4.27 min to 58.3 +/- 4.98 min[24]. Unlike other studies[25,26,28], these investigators also could not demonstrate differences in the PK of ampicillin between non-pregnant individuals and pregnant women before the onset of labor[24]. In contrast to this study, we did not find differences in terminal half-life of amoxicillin between women before and after the onset of labor. Differences in study design might explain the discrepancy between the results. In the ampicillin study four blood samples were collected from each patient, whereas in our study an average of 18 blood samples was obtained for each patient per stage of labor. More intensive sampling will result in more reliable estimates of the inter-individual variability. The pharmacokinetic description of studies with a high sampling density are expected to be more accurate and harbor the possibility of detecting also small differences in pharmacokinetic parameters between various patients. Whether differences in methodology or true differences between the study populations underlies the different conclusions of the studies remains to be determined. Alternatively, differences in chemical structure or features of the two drugs might also explain differences in the results. However, since the pharmacokinetics of ampicillin and amoxicillin after intravenous administration in non-pregnant individuals has been shown to be similar in previous studies[29,30], this is unlikely.

Clinically relevant pharmacokinetic changes during pregnancy have also been demonstrated for other drugs. For example, the plasma concentration of the antiepileptic drug lamotrigine has been shown to decrease during pregnancy. Subsequently, in the immediate postpartum period, the plasma concentration increases rapidly, resulting in a risk for toxicity[31]. This indicates that changes of specific drugs during pregnancy and labor cannot be readily extrapolated on the basis of results obtained with other drugs.

The inter-individual variability in amoxicillin pharmacokinetics between the patients was remarkably moderate. In figure 1 the observed concentrations are plotted versus the population predicted concentrations. The majority of the data points are located near the line of identity. However, for some observed concentrations the predicted concentrations are overestimated. These blood samples were taken during the antibiotic infusion. The antibiotic concentration increases very fast during the infusion. We recorded the sampling times with a precision of 0.5-1 minute. Therefore, the model will predict concentrations during the antibiotic infusion less accurately compared to concentrations in blood samples collected after the antibiotic infusion.

An important question for the clinical practice is whether the recommended amoxicillin dosing regimen is adequate in the prevention of GBS disease. The

efficacy of amoxicillin is determined by the time the concentration exceeds the minimum inhibitory concentration (T>MIC) and, in general, T>MIC for 40-50 % of the dosing interval is required for efficacy[32-34]. The MIC value of an antibiotic is the highest MIC value of different microbial strains that results in a high probability of cure. MIC value of amoxicillin for GBS as indicated by the EUCAST is 0.25 mg/L[35]. All concentrations remained well above the MIC of GBS for a sufficient percentage of the dosing interval, even when taking into account the plasma protein binding of approximately 18-20 %[36,37].

After delivery, the pregnancy-induced physiological adaptations will change back to normal. This process starts immediately postpartum, but will continue for several weeks[38,39]. In the first days after delivery, this includes an increase in blood volume and cardiac output. We found only minor differences in pharmacokinetic behavior of amoxicillin in the immediate postpartum period when compared to pregnant women. This supports our earlier study that demonstrated a similar PK of amoxicillin in pregnant women with PPROM compared to values reported for non-pregnant individuals.

In our study, in none of the patients toxic or sub-therapeutic concentration-profiles were reached. For this reason the differences in PK in the three stages of labor were considered not clinically relevant. This finding supports the current practice that the dosing regimen is not adjusted during the course of labor. However, it should be noted that we included only healthy patients and that only 8 patients were included both before and during labor. Our patient group may be considered to be small, but taking into account the situation in which the patients had to be studied this is a relatively large population. Antibiotics in the prevention of GBS are also used to protect the fetus. Therefore, adequate fetal concentrations are imperative. Since uterine contractions might influence the blood flow in the umbilical cord, studies investigating the transplacental transfer of amoxicillin during vaginal deliveries are needed to describe the PK in umbilical cord serum and ultimately in the fetus.

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Chapter 6

Clavulanic acid does not influence amoxicillin pharmacokinetics in pregnant women during labor.

Anouk E. Muller, Rob A. Voskuyl, Johan W. Mouton, Paul M. Oostvogel, Joost DeJongh, Eric A.P. Steegers, Meindert Danhof, P. Joep Dörr

Abstract

We studied the pharmacokinetics of amoxicillin when co-administered with clavulanic acid in pregnant women during labor. Co-administration of amoxicillin with clavulanic acid did not result in changes in amoxicillin pharmacokinetics. The mean values (+/- SD) of the estimates for the CL of amoxicillin in patients treated with amoxicillin and amoxicillin in combination with clavulanic acid were, respectively, 22.8 +/- 2.4 L/h and 21.3 +/- 6.9 L/h. For V_1 the mean values (+/- SD) of the estimates were 11.0 +/- 2.6 L and 13.4 +/- 7.0 L for patients treated with, amoxicillin with and without clavulanic acid respectively. Based on these pharmacokinetic findings dose adjustments for amoxicillin during labor are not necessary when the drug is co-administered with clavulanic acid.

Introduction

Augmentin® or co-amoxiclav, an antibiotic formulation of amoxicillin and clavulanic acid, is widely used in pregnant women as treatment of choice for intra-amniotic infection (IAI). IAI during delivery is a serious complication and affects both mother and neonate. This can occur in up to 10.5 percent of all deliveries[1-5]. The frequency of IAI is highest in preterm deliveries[6].

When amoxicillin and clavulanic acid are administered simultaneously, the activity spectrum of amoxicillin against both gram-positive and gram-negative bacteria is enhanced. Clavulanic acid irreversibly inhibits a wide range of β-lactamases produced by micro-organisms, thereby protecting the β-lactam antibiotics from enzymatic inactivation[7]. The intrinsic antibacterial activity of clavulanic acid alone is negligible[7].

Previously, several other studies have reported that the pharmacokinetic behavior of amoxicillin in healthy volunteers and children is not modified by the co-administration of clavulanic acid[8-11]. On the other hand, one study administering varying doses of oral amoxicillin together with 125 mg clavulanic acid, showed that at the highest amoxicillin dose (875 mg) clavulanic acid absorption was reduced while amoxicillin absorption was unaffected[12]. Dose-dependency in the pharmacokinetics of amoxicillin has also been described in other studies[13,14].

Thus, it cannot be excluded that under certain circumstances co-administration of clavulanic acid might influence the concentration-time profile of amoxicillin. Potential interactions between simultaneously administered drugs might be caused by inhibition of metabolic enzymes, competition for protein binding in plasma or changes in renal transporter-systems. Consideration of the elimination routes of the simultaneously administered drugs is therefore important to assess the probability of drug interactions. Both amoxicillin and clavulanic acid are eliminated by hepatic metabolization and renal excretion. Amoxicillin is in part metabolized to the inactive compound penicilloic acid and is eliminated by the kidneys. Clavulanic acid is also partly metabolized and in animal studies it has been shown that the metabolites are excreted by feces, urine and lung secretions[15,16]. Physiological changes taking place during pregnancy and labor, such as an increase in cardiac output and glomerular filtration rate[17-19], may also influence the pharmacokinetics of (co-administered) drugs. These adaptations may include changes in protein binding and the function of and/or blood flow to the metabolizing and eliminating organs. The aim of this study was to investigate whether co-administration of clavulanic acid influences the pharmacokinetics of amoxicillin in pregnant women during labor.

Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women who needed antibiotic treatment with amoxicillin or co-amoxiclav during labor and were admitted to the hospital, were eligible for inclusion in this study. Following local guidelines, women were treated with amoxicillin in the prevention of GBS, in case of a proven or unknown *Streptococcus agalactiae* (group B streptococcus, GBS) carriage, in the presence of generally recognized risk factors for neonatal GBS disease but without signs of infection[20]. In case of suspected intra-amniotic infection, women were treated with co-amoxiclav. The study was approved by the Medical Ethics Committee of the Medical Center Haaglanden, the Hague, the Netherlands. Written informed consent was obtained from all patients. Women were excluded from the study if they i) had been treated with oral or intramuscular antibiotics within 2 days before starting therapy, ii) were unwilling to comply with the requirements of the study, iii) were known to be allergic to amoxicillin or other penicillins, or iv) were receiving co-medication that exhibits interaction with amoxicillin. At the start of the study all patients were at least 18 years of age and in labor.

Drug administration and blood sampling

Before the administration of amoxicillin or co-amoxiclav an intravenous catheter was placed in each arm. The choice of the antibiotic and the dosing schedule was in accordance to the guidelines of the local hospital. Treatment with amoxicillin started with an intravenous infusion of 2 gram amoxicillin (50 mg/mL) administered over 30 minutes, followed by a second infusion after 4 hours of 1 gram amoxicillin (50 mg/mL) over 15 minutes. Treatment with co-amoxiclav (consisting of 1 gram amoxicillin (50 mg/mL) with 200 mg clavulanic acid) consisted of an infusion for 15 minutes every 8 hours. Blood samples of 2 mL were collected from the second catheter in the contra-lateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 7 and 15 min (1 gram infusion) or 15 and 30 min (2 gram infusion) during the first two amoxicillin administrations. After the infusion sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30 minutes until the next antibiotic dosage. Exact sampling times were recorded.

All blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 minutes. The supernatants were transferred into plastic storage tubes and frozen at –70° C until analysis.

Patient information

All patients received a standard work-up at the onset of the study which included a medical history, biochemical and hematological examination. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded. The amount of edema was scored semi-quantitatively from 0 (no edema), 1 (around the ankle), 2 (up to the knee) to 3 (above the knee).

High-performance liquid chromatography

Amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatography (HPLC) (Shimadzu, Den Bosch, The Netherlands (NL)) method, using an ODS Gemini column (Bester, Amstelveen, NL) with 0.066 M KH2PO4 solution containing 10 % methanol as a mobile phase. A perchloric acid solution of 0.1 ml was added to the sample in an equal volume and after vortexing, added to 0.56 ml 0.028 M citric acid containing cefadroxil (Sigma, Zwijndrecht,NL) as an internal standard. The assay was linear over the concentration range measured. Controls were included in every run. The lower limit of detection was 0.2 mg/L and the between run CV < 4%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect (population) Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM® software package (version VI, release 1.2, ICON Development Solutions, Ellicott City, USA).

The data of all patients were analyzed simultaneously to determine the basic structural pharmacokinetic parameters. Various 2- and 3- compartment models were tested. The previously described pharmacokinetic population model in pregnant women with preterm premature rupture of the membranes (PPROM) consisting of 3 compartments, was used to start the analysis[21]. Model selection and identification of variability was based on the objective function value (OFV), pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. To estimate the structural parameters, a significance level of 0.001 was selected (corresponding to differences in OFV of at least 10 points). NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits the goodness-of-fit plots were visually inspected. The data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should visually be randomly distributed around zero. Population values were

estimated for the parameters clearance (CL), the volumes of distribution (V) and the intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log normal distribution. Therefore an exponential distribution model was used to account for inter-individual variability. Possible correlation between inter-individual variability coefficients on parameters was estimated and if present accounted for in the stochastic model (NONMEM Omega block option).

Selection of an appropriate residual error model was based on the OFV, their respective confidence intervals and inspection of the goodness-of-fit plots. A additive error model, proportional error model and a combined additive and proportional error model were evaluated. The residual error term contains all the error terms which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters were plotted independently against the covariates bodyweight, body mass index, gestational age, creatinin, leucocytes, oral temperature, and the amount of edema to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance using the OFV and the residual intra- and inter-individual variability were visually evaluated. A significance level of 0.05 was selected, corresponding to differences in OFV of 3.84 points. A further criterion for acceptance of the covariate effects is that the estimated 95% confidence interval of the covariate effect did not overlap with it's null value.

The effect of co-administration of clavulanic acid on structural amoxicillin PK parameters was investigated using the entire population as one group. The difference between treatment with amoxicillin alone of co-amoxiclav was implemented in the model as covariate on the structural parameters with significant inter-individual variability. The effect of co-administration of clavulanic acid was evaluated using the OFV with a significance level of 0.05, the 95% confidence interval and the goodness-of-fit plots. The residual intra- and inter-individual variability were visually evaluated.

The accuracy of the final population model for the entire population was established using a bootstrap method in NONMEM, consisting of repeated random sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

Results

Eighteen patients were included in the study. Nine patients were treated with co-amoxiclav and 9 with amoxicillin alone. In total 218 serum samples were collected

in the study, 94 samples from patients treated with co-amoxiclav and 124 samples from patients treated with amoxicillin. All samples were used as a part of other study groups[22, 23]. The gestational age of patients treated with amoxicillin was lower (35.3 weeks) compared to patients treated with co-amoxiclav (39.8 weeks). Of the patients treated with co-amoxiclav 5 patients had a temperature above 37.8ºC, compared to 2 patients treated with amoxicillin. 9 patients were nulliparae. Of the patients receiving co-amoxiclav 2 delivered after a secondary caesarean section. All patients treated with amoxicillin delivered vaginally. The characteristics of the study patients are presented in table I.

		Patients treated with amoxicillin			**Patients treated with co-amoxiclav**		
Data	**units**	**mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Maternal age	year	30.0	6.9	21.2-37.9	31.6	4.2	24.9-38.5
Gestational age	week	35.3*	2.6	31.0-39.1	39.8*	1.9	36-42.4
Weight	kg	73.4	10.8	53.0-87.3	81.3	16.2	56-108
Body Mass Index	kg/m^2	26.0$^{\nabla}$	4.3	18.3-32.0	31.7$^{\nabla}$	6.0	24.2-41.6
Leucocytes	$x10^9/L$	16.4	6.4	9.4-28.2	14.3	4.8	8.4-23.1
Creatinin	µmol/L	45.8	7.6	35-56	56.3	17.8	34-89
Temperature	ºC	36.9	1.1	35.4-39.1	37.7	0.6	36.7-38.6
Edema**		1.22	0.4	1-2	1.78	0.7	1-3

SD: standard deviation; The unpaired t-test was used to detect significant differences between patients treated with amoxicillin and patients treated with co-amoxiclav. * significant difference ($p<0.001$); significant difference ($p<0.05$) ** The amount of edema was score as: no (0) /around the ankle (1) /up to the knee (2) /above the knee (3).

Table 1*: characteristics of the study patients.*

A 3-compartment model best described the data. The inter-individual variability was mainly due to differences in CL and V_1. A correlation between the random parameters for inter-individual variability was not found. An increase of V_1 of 2.1% per kg body weight gain was found and incorporated into the model. Implementation of the other covariates did not improve the model-fit. The residual error was found to be proportional to the blood concentrations. The randomly distributed observed concentrations versus model-predicted concentrations as shown in figure 1, illustrate the unbiased model fit. For some observed concentrations the predicted concentrations are overestimated. These blood samples were taken during the

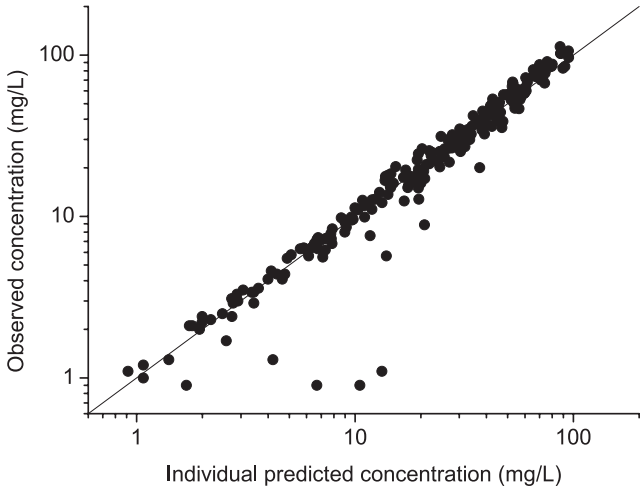

***Figure 1**: observed versus individual predicted concentrations.*

Parameter	Units	Mean	SE
Structural model parameters			
CL	L/h	21.3	1.58
V_1	L	11.8	1.3
V_2	L	5.72	1.01
V_3	L	8.01	1.52
Q_1	L/h	28.2	7.89
Q_2	L/h	5.4	1.37
Variance model parameters			
Interindividual variability in CL		0.080	0.051
Interindividual variability in V_1		0.055	0.017
Residual variability		0.040	0.0092

CL Clearance; V_1 Volume of distribution of the central compartment; V_2 volume of distribution of the first peripheral compartment; V_3 volume of distribution of the second peripheral compartment; Q_1 intercompartmental clearance between V_1 and V_2; Q_2 intercompartmental clearance between V_1 and V_3.

***Table 2:** Parameter estimates of the final model.*

antibiotic infusion. The antibiotic concentration increases very fast during the infusion. We recorded sampling times with a precision of 0.5-1 minute. Therefore, the model will predict concentrations during the infusion less accurately compared to concentrations obtained after the infusion. Table 2 shows the estimated values for the pharmacokinetic parameters of the final model. The parameter estimates obtained with the bootstrap analysis did not differ significantly from the parameters estimated from the final model (99 of 100 runs successful, data not shown).

Co-administration of clavulanic acid to the amoxicillin did not influence the pharmacokinetics of amoxicillin during labor after intravenous drug administration. The OFV decreased with 1.01 and 0.15 points after implementation of the covariate for the difference between the drugs on CL and V_1, respectively. This is supported by the individual estimates of CL and V_1, as generated by NONMEM. The mean values (+/- SD) of the estimates for the CL of amoxicillin in patients treated with

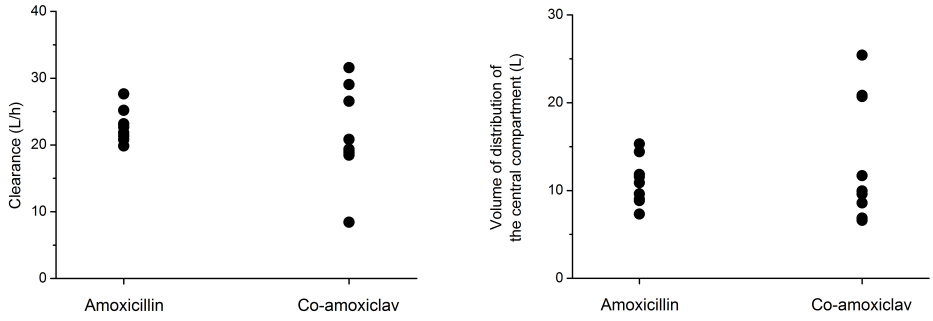

Figure 2:
Distribution of clearance (figure 2A) and central volume of distrubution (figure 2B) for patients receiving amoxicillin and co-amoxiclav.

amoxicillin and co-amoxiclav were, respectively, 22.8 +/- 2.4 L/h and 21.3 +/- 6.9 L/h. For V_1 the mean values (+/- SD) of the estimates were 11.0 +/- 2.6 L and 13.4 +/- 7.0 L for patients treated with amoxicillin and co-amoxiclav, respectively. Figure 2A and 2B show the distribution of the structural parameters CL and V_1 for patients treated with amoxicillin and patients with co-amoxiclav.

Discussion

Pharmacokinetics of amoxicillin in pregnancy has been described previously and it has been shown that adherence to the guidelines leads to adequate concentration-time profiles in the mother in the prevention of GBS[21]. However, as co-administration of other drugs might influence the concentration-time profile of an individual drug, we investigated in this study the frequently used alternative, the combination of amoxicillin and clavulanic acid. In this study, we show that the pharmacokinetics of intravenously administered amoxicillin during labor is not significantly influenced by co-administration of clavulanic acid.

A number of potential interactions had been anticipated. Both drugs bind to serum proteins and are eliminated via the same routes. Distribution might be influenced by competition of the drugs for the same serum proteins. This might in principle increase the unbound fraction of the drug, resulting in a different tissue penetration and a higher rate of biotransformation and excretion. However, since both amoxicillin and clavulanic acid have low plasma protein binding of respectively 20% and 22%[24], the probability that this would lead to a significant change after the administration of co-amoxiclav is small. Inhibition or induction of enzymes involved in metabolism might also have caused changes in pharmacokinetics of the drugs. Both amoxicillin and clavulanic acid are in part metabolized, but the absence of pharmacokinetic drug interaction in this study indicates that the individual drugs do not influence the enzyme activity involved in each others metabolism. Both changes in passive and active excretion of drugs could have caused drug interaction. Passive re-absorption may change due to a change in the pH of the urine and on the other hand, transporters involved in the active excretion of drug may be influenced. Our results indicate that administration of co-administration of clavulanic acid with amoxicillin did not significantly influence the distribution, metabolization and excretion of amoxicillin.

Data on the pharmacokinetics of drugs in pregnant patients are difficult to obtain both for ethical and practical reasons. Therefore, comparison of different patient groups and discovering sources of variability is nearly impossible by using conventional statistical methods. Particularly in this type of studies the use of population modeling is an advantage, because covariate analysis applied to the whole group of patients maximizes the utilization of all information that is available for each patient. The finding that body weight has a slight influence on clearance illustrates this point. Furthermore, if data of more patients would became available, this can be simply added to the dataset, without the need to carry out a study with new groups of patients.

In the analysis of the pharmacokinetics of amoxicillin in this patient group the volume of distribution of the central compartment increased with body weight. In an earlier study the volume of distribution of the central compartment increased

with an advancing gestational age[23]. Because gestational age is correlated with body weight ($p<0.01$), this is not a discrepancy between these analysis. The use of amoxicillin is indicated in patients during a preterm delivery. Consequently, the gestational age in patients treated with amoxicillin is lower than in patients treated with co-amoxiclav. The increasing body mass index increases with advancing gestational age, will also result in a significant difference in body mass index between the two groups. The effect of covariates on the PK is investigated in the entire patient group and these differences will therefore not influence the results.

A proper dosing regimen of amoxicillin is essential for the treatment of IAI in pregnant women. Co-administration of clavulanic acid to amoxicillin increases the antibacterial spectrum of the treatment. Different dose ratios of amoxicillin/clavulanic acid had been used. But since the higher doses of clavulanic acid was associated with a higher rate of adverse effects, such as diarrhea, nausea and vomiting, the amount of clavulanic acid was decreased[16]. Data on the pharmacokinetics of clavulanic acid during labor are not available. Unfortunately, the concentrations of clavulanic acid could not be determined in this study due to stability issues. As there are no reports that the efficacy of the these dose ratio's are different, the amount of clavulanic acid may not be that critical[12].

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Chapter 7

Pharmacokinetics of amoxicillin in maternal, umbilical cord and neonatal serum.

Anouk E. Muller, Paul M. Oostvogel, Joost DeJongh, Johan W. Mouton, Eric A.P. Steegers, P. Joep Dörr, Meindert Danhof, Rob A. Voskuyl

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Abstract

The pharmacokinetics of amoxicillin was studied in umbilical cord and neonatal serum relative to maternal concentrations in prevention of neonatal Group B Streptococcus infection. Subjects were 44 pregnant women receiving amoxicillin as 1 or 2 gram intravenous infusion. To measure concentrations, blood samples were obtained from the mother, arterial and venous umbilical cord and neonate. The pharmacokinetics were characterized by a 5-compartment model using nonlinear mixed-effects (population) modeling. The population estimates for the clearance, central volume of distribution and the 2 peripheral maternal volumes of distribution 19.7 +/-0.99 L/h, 6.40+/-0.61 L, and 5.88+/-0.83 L, (mean +/- standard error), respectively. The volume of distribution of the venous umbilical cord and the neonatal volume of distribution were 3.40 L and 11.9 L. The pharmacokinetic parameter estimates were used to simulate concentration-time profiles in maternal, venous umbilical cord and neonatal serum. The peak-concentration in venous umbilical cord serum was 18% of the maternal peak-concentration. It was reached 3.3 minutes after the maternal peak-concentration. The concentration-time profile in neonatal serum is determined by the profile in venous umbilical cord serum, which in turn depends on the profile in maternal serum. Furthermore, the simulated concentrations in maternal, venous umbilical cord and neonatal serum exceeded the minimal inhibitory concentration for group B streptococcus for more than 90% of the 4 hour dosing interval. In first approximation, the 2 gram infusion to the mother appears to be adequate in the prevention of group B streptococcal disease. However, to investigate the efficacy of the prophylaxis, further studies on the inter-individual variability in pharmacokinetics are indicated.

Introduction

Amoxicillin, a penicillin derivative, is an antibiotic used in the prevention of neonatal group B streptococcal (GBS) disease. Neonates from mothers colonized with GBS are at risk for vertical transmission, because they might be exposed to GBS in utero or in the vagina during delivery. While protection of the fetus is the actual objective of the prophylaxis, the procedure in GBS prophylaxis is to administer the antibiotics to the pregnant woman. Since antibiotics reach the fetus only after transport over the placenta via the umbilical cord, adequate concentrations in maternal serum are a prerequisite, but no guarantee, for adequate venous and arterial umbilical cord and fetal serum concentrations. While there are some data on ampicillin concentrations in umbilical cord blood[1,2], data for amoxicillin are not available. The pharmacokinetics of amoxicillin in pregnant women before labor have been described previously and have been shown to exceed the MIC for an adequate percentage of the dosing interval for treatment of the infection in the mother[3]. To assess whether the administration of amoxicillin also protects the fetus from GBS infection, data on the pharmacokinetics in umbilical cord serum or fetal serum are necessary.

Studying the pharmacokinetics in fetal or umbilical cord serum faces ethical and practical difficulties. Because blood sampling of the fetus during delivery is not possible, blood samples of the umbilical cord taken after birth is in most instances the only direct information on fetal concentrations that can be obtained. Unfortunately, these samples can be obtained only at a single point in time for each individual. Alternatively, blood samples of the neonate taken by heel puncture shortly after birth can be considered as a good approximation of the fetal concentration. However, such samples are difficult to obtain because of the poor blood supply to the extremities directly after birth. When neonatal blood samples are taken hours later, these concentrations might not truly reflect the fetal concentrations, because of the differences in the rates and routes of elimination before and after birth.

By necessity the time intervals between the last antibiotic dose and birth will be different for each individual. Therefore presenting either individual or average concentrations in the umbilical cord samples at birth will be of little value. To obtain useful information the pharmacokinetics (i.e. the concentration versus time profile) in the umbilical cord serum should be known. For this purpose population pharmacokinetic modeling is useful, because in this approach all data of the entire study population (i.e. all mothers and fetuses) are taken into account. An important feature of population pharmacokinetic modeling is that it enables the analysis of data from studies with unbalanced designs (e.g. unequal groups) and of incomplete datasets[4,5]. Specifically, Non-Linear Mixed Effects ('population') modeling can be used to connect the sparse data on umbilical cord and neonatal blood concentrations

to the more detailed information on the pharmacokinetics in the mother. A more detailed background of population modeling can be found elsewhere[6,7]. Recently a population pharmacokinetic model of amoxicillin in pregnant women has been proposed[3]. The objective of this study is to describe the concentration-time profile of amoxicillin in umbilical cord and neonatal serum, in relation with the concentration-time profile in maternal serum.

Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women who needed antibiotic treatment with amoxicillin or amoxicillin/clavulanic acid (Augmentin® or co-amoxiclav) shortly before or during labor were eligible for this study. To take full advantage of all data available to us for the development of a population pharmacokinetic model of amoxicillin in pregnant women, umbilical cord and neonate, the present study includes part of a dataset of a recently published study[3]. 416 blood samples from pregnant women with preterm premature rupture of the membranes were used in a previous study. None of the samples of the umbilical cord or the neonate were previously used. Following local guidelines, women were treated with amoxicillin in the prevention of GBS disease, when no signs of infection were present, but with proven or unknown *Streptococcus agalactiae* carriage, in the presence of generally recognized risk factors for neonatal GBS disease[8]. In case of suspected intra-amniotic infection, women were treated with co-amoxiclav. When signs of infection were present delivery was induced. The study was approved by the Medical Ethics Committee. Written informed consent was obtained from all patients. Women were excluded from the study when i) they had been treated with oral or intramuscular antibiotics within 2 days before starting therapy, ii) were unwilling to comply with the requirements of the study, iii) were known to be allergic to amoxicillin or other penicillins, or iv) were receiving co-medication that exhibits interaction with amoxicillin. All patients were at least 18 years of age.

All patients received a standard work-up which included a medical history, biochemical and hematological examination. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded. The amount of edema was scored semiquantitatively from 0 (no edema) to 3 (above the knee). The weight of the placenta with umbilical cord was measured. From the neonate birth weight and the Apgar-scores after 1, 5 and 10 minutes were recorded.

Drug administration and blood sampling

Before the administration of amoxicillin or co-amoxiclav an intravenous catheter was placed in each arm. Antibiotics were administered following local guidelines.

Treatment with amoxicillin started with an intravenous infusion of 2 gram amoxicillin (50 mg/mL) administered over 30 minutes, followed by a second infusion after 4 hours of 1 gram amoxicillin (50 mg/mL) over 15 minutes. Treatment with co-amoxiclav (consisting of 1 gram amoxicillin (50 mg/mL) with 200mg clavulanic acid) consisted of an infusion for 15 minutes every 8 hours. Blood samples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 7 and 15 min (1 gram infusion) or 15 and 30 min (2 gram infusion) during the first two amoxicillin administrations. After the infusion sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30 minutes until the next antibiotic dosage. Exact sampling times were recorded.

After cord clamping the umbilical cord was cleaned with normal saline and a sponge to prevent contamination of maternal blood in the umbilical cord samples. Both arterial and venous cord samples of 5-10 mL were taken. From the neonate a blood sample of approximately 0.5 mL was obtained by heel puncture after signed informed consent from both parents. These blood samples were taken at least 10 minutes after birth depending on the physical condition of the neonate.

All blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 minutes. The supernatants were transferred into plastic storage tubes and frozen at –70° C until analysis.

High-performance liquid chromatography

Amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatography (HPLC) (Shimadzu, Den Bosch, The Netherlands (NL)) method, using an ODS Gemini column (Bester, Amstelveen,NL) with 0.066 M KH_2PO_4 solution containing 10% methanol as a mobile phase. A perchloric acid solution of 0.1 ml was added to the sample in an equal volume and after vortexing, added to 0.56 ml 0.028 M citric acid containing cefadroxil (Sigma, Zwijndrecht,NL) as an internal standard. The assay was linear over the concentration range measured. Controls were included in every run. The lower limit of detection and the lower limit of quantification were 0.2 mg/L and the between run CV < 4%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect (population) Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method with INTERACTION. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM® software package (version VI, release 1.2, ICON Development Solutions, Ellicott City, Maryland, USA).

To determine the basic structural pharmacokinetic parameters various 3-, 4- and 5- compartment models were tested. The previously described 3 compartment pharmacokinetic population model in pregnant women with PPROM, was used to describe the time course of amoxicillin in maternal blood[3]. Model selection and identification of variability was based on the likelihood ratio test, pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For the likelihood ratio test on differences between two models, the objective function value (OFV) with a pre-specified level of significance of $P<0.001$ was used. NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits the goodness-of-fit plots were visually inspected. The data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero. Population values were estimated for the parameters clearance (CL), the volumes of distribution (V) and the intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log normal distribution. Therefore an exponential distribution model was used to account for inter-individual variability. The correlations between the various random parameters for inter-individual variability were tested using the forward addition and backward deletion method in the NONMEM Omega block option.

Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots. The residual variability between the observed concentrations and those predicted by the model was described using a proportional error model. The residual error term contains all the error terms which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters were plotted independently against the covariates bodyweight, body mass index, duration of amenorrhea, blood pressure, pulse, oral temperature, and the amount of edema to determine whether this influenced the pharmacokinetics. Covariate analysis was performed by forward addition of each candidate covariate into the model structure until no further improvement of the goodness of fit was observed. A further criterion for acceptance of covariate effects was that the estimated confidence interval of the covariate effect did not overlap zero. Contribution of each covariate to the final model was confirmed by backward elimination of each covariate from the model to account for possible interaction between covariates. The residual intra- and inter-individual variability were visually evaluated.

The accuracy of the final population model for the entire population was established using the bootstrap option in NONMEM, consisting of repeated random

sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

Simulations

Simulations were performed to determine the relation in time between the maternal, umbilical cord and neonatal serum concentration-time profiles. The simulations were performed using Berkeley Madonna (version 8.3.5, Berkeley Madonna Inc, University of California, USA). The mean population parameter values of the pharmacokinetic model derived with NONMEM in the first part of the analysis were used in the simulations.

Results

From 44 pregnant patients 53 umbilical cord blood samples were obtained, consisting of 25 arterial and 28 venous samples. Of 23 women both arterial and venous samples were obtained. Four umbilical cord samples of one twin pregnancy were collected. A total of 904 maternal serum samples were collected. Due to the unpredictable and variable duration of labor and to the varying emotional condition of the patient, the number of maternal samples obtained from each patient is variable (range 3-41 samples per patient). The time interval between the last antibiotic dose and birth varied from 24.4 min to 316.8 min. The patients gave birth at gestational ages from 30.0 to 42.4 weeks. Birth weight of the neonates ranged from 1340 gram to 4470 gram. Fourteen blood samples were taken from the neonates between 14.2 min to 199.8 min after birth. The characteristics of the study patients and their neonates are presented in table I.

The concentrations in cord serum range from 1.0-16.8 mg/L in arterial and 1.1-18.0 mg/L in venous umbilical cord serum. The ratio of the arterial and venous umbilical cord serum concentrations is shown in figure 1. The ratio was <1 when the umbilical cord was clamped shortly after the administration of the last antibiotic dose and slightly higher with an increasing interval between the last antibiotic administration and umbilical cord clamping. The differences between the arterial and venous umbilical cord serum concentrations were too small to analyze these concentrations using two separate compartments. Since arterial cord blood originate directly from the fetal circulation, the arterial cord blood concentrations can be considered as fetal blood concentration. Venous cord blood concentrations reach the umbilical cord after passage of the placenta. Therefore these concentrations were analyzed separately.

In population modeling data of concentrations in maternal, arterial and venous umbilical cord, and neonatal serum were analyzed simultaneously. Of all

Maternal patient characteristics	units	mean	SD	Number of patients
Maternal age	y	30.0	6.85	44
Maternal weight	kg	79.4	13.9	43
Body Mass Index	kg/m^2	29.4	5.3	43
Edema (no/around the ankle/up to knee)	-	-	-	29/12/3
Leucocytes	x 10^9/L	12.8	5.14	44
Creatinin	µmol/L	47.4	11.5	44
Amenorrea	weeks	36 6/7	2.7	44
Nulliparity	-	-	-	22
Twin pregnancy	-	-	-	3
Mode of delivery (vaginal/vacuum extraction/ emergency caesarean section)	-	-	-	23/1/3*
Placental weight	gram	546.5	165.0	40
Birth weight neonate	gram	2887.4	627.9	46
Apgar score 1 min	-	8.7	1.05	41
Apgar score 5 min	-	9.6	0.84	41
Apgar score 10 min	-	9.8	0.59	40

* Only patients from whom umbilical cord serum was obtained.

***Table I**: characteristics of the study patients, placental weight and their neonates*

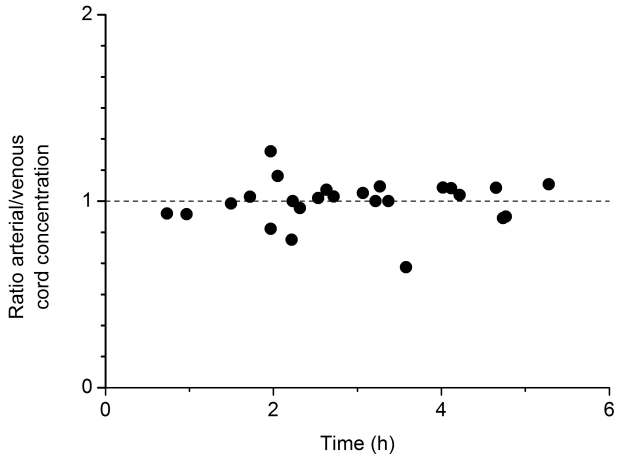

***Figure 1**: ratio of arterial and venous umbilical cord serum concentrations as function of the time.*
The ratio of the arterial and venous umbilical cord serum concentrations was plotted versus the time interval between the last antibiotic administration to the mother and child birth.

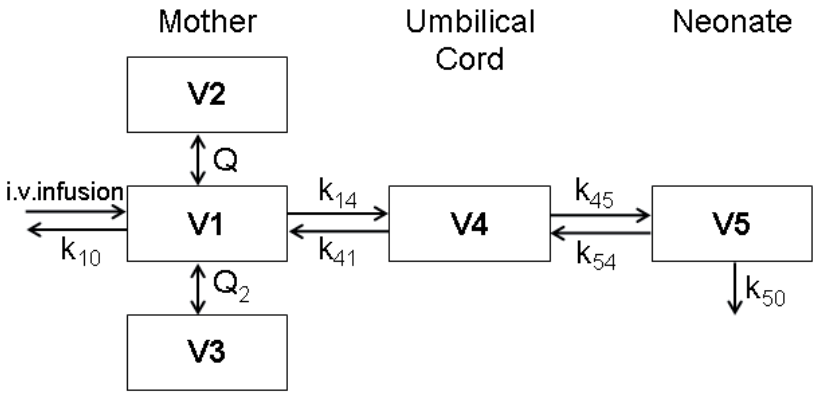

***Figure 2:** 5-compartment model*
Structure of the final 5-comparment model consisting of a central volume of distribution of the mother (V_1), peripheral volumes of distribution of the mother (V_2 and V_3), a volume of distribution of the umbilical cord (V_4) and a volume of distribution of the neonate (V_5). The k-values represent the intercompartmental exchange rate constants.

models tested a multicompartment pharmacokinetic model with three compartments for the mother, 1 compartment for the venous umbilical cord, and 1 compartment for the neonate best described the data (figure 2). The peripheral compartments 2, 3 and 4 were connected to the central compartment. Compartment 5 is attached to compartment 4 and antibiotics in compartment 5 are transferred back to compartment 4, and eliminated from the system. During the analysis the values of the volumes of distribution V_2 and V_3 of compartment 2 and 3 were comparable (difference in the final model with separate estimates for V_2 and V_3 <0.5 L). The %CV of these estimates exceeded 51% and therefore for the final analysis V_3 was assumed to be equal to V_2. The inter-individual variability was mainly due to differences in clearance, V_1 and the residual error. A correlation between the random parameters for inter-individual variability was found and accounted for in the model. The residual error was found to be proportional to the blood concentrations.

The demographic and clinical characteristics of the patients were examined as potential covariates on the parameters CL, V_1, K_{45} and the residual error. According to the specified OFV change criterion, gestational age (ΔOFV = - 10.5) and body mass index (ΔOFV = - 8.5) both influenced the volume of distribution. Although a correlation was noted between gestational age and body mass index, incorporation of these two covariates on the volume of distribution did not result in a significant decrease in OFV (ΔOFV = - 3.3). Because the decrease in OFV was larger after incorporation of gestational age then after incorporation of body

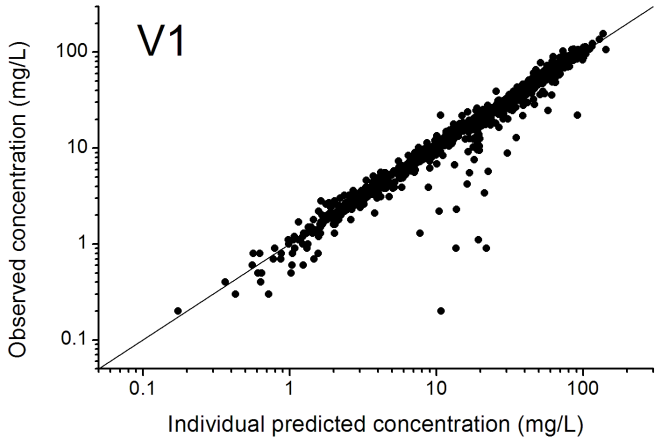

Figure 3: *Individual predicted vs observed concentrations of amoxicillin for the central compartment of the mother (V_1), the umbilical cord (V_4) and neonatal compartment (V_5).*

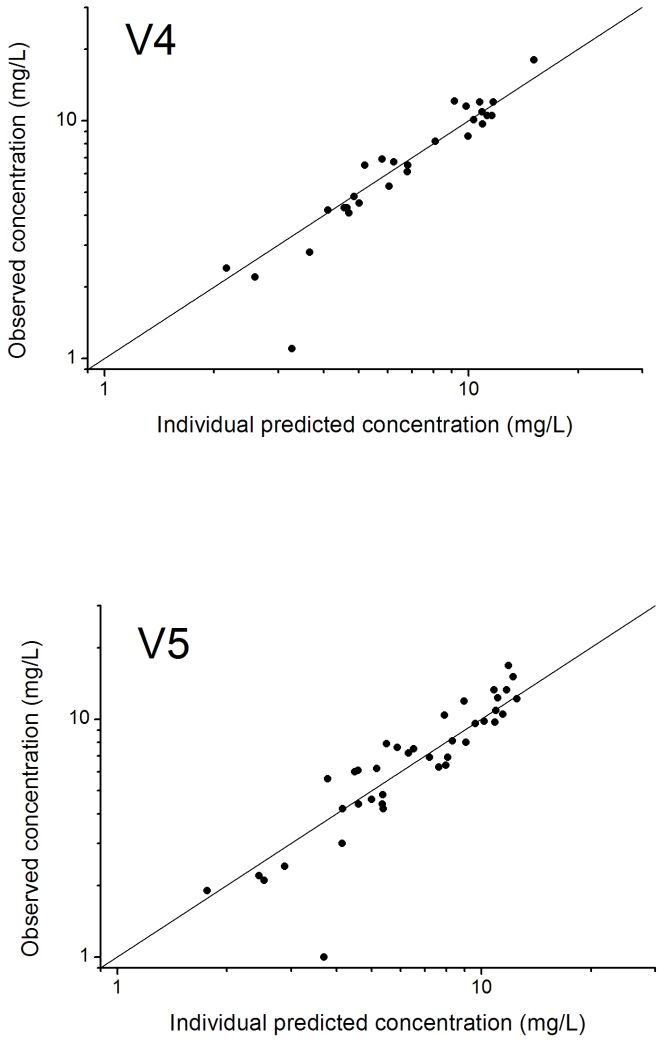

Figure 3: *Individual predicted vs observed concentrations of amoxicillin for the central compartment of the mother (V_1), the umbilical cord (V_4) and neonatal compartment (V_5). Scatter plots of the individual predicted vs observed concentrations of amoxicillin for 44 patients (V_1), 53 measures of the umbilical cord (V_4) and 14 neonatal measures (V_5). The figures show the individual data points and the line of identity (x=y).*

Parameter	Units	Mean	SE	95% confidence interval
Structural model parameters				
CL	L/h	19.7	0.99	17.8 – 21.6
V_1	L	6.4	0.61	5.2 – 7.6
V_2	L	5.88	0.83	4.2 - 7.5
V_3	L	5.88	0.83	4.2 – 7.5
Q_1	L/h	56.6	9.5	38.0 – 75.2
Q_2	L/h	10.7	2.2	6.3 – 15.1
K_{14}		0.76	0.28	0.21 – 1.3
K_{41}		1.4	0.31	0.83 – 2.1
K_{45}		5.1	2.0	1.1 – 9.1
K_{54}		1.4	0.31	0.83 – 2.1
K_{50}		0.16	0.033	0.098 – 0.23
Variance model parameters				
Interindividual variability in CL		0.076	0.026	0.026 – 0.13
Interindividual variability in V_1		0.038	0.013	0.014 – 0.063
Residual variability		0.030	0.0040	0.022 – 0.037

***Table 2**: Population model parameter values.*
CL: clearance, V_1: volume of distribution of the central compartment, V_2: volume of distribution of the first peripheral compartment, V_3: volume of distribution of the second peripheral compartment, Q_1: intercompartmental clearance between V_1 and V_2, Q_2: intercompartmental clearance between V_1 and V_3, SE: Standard error

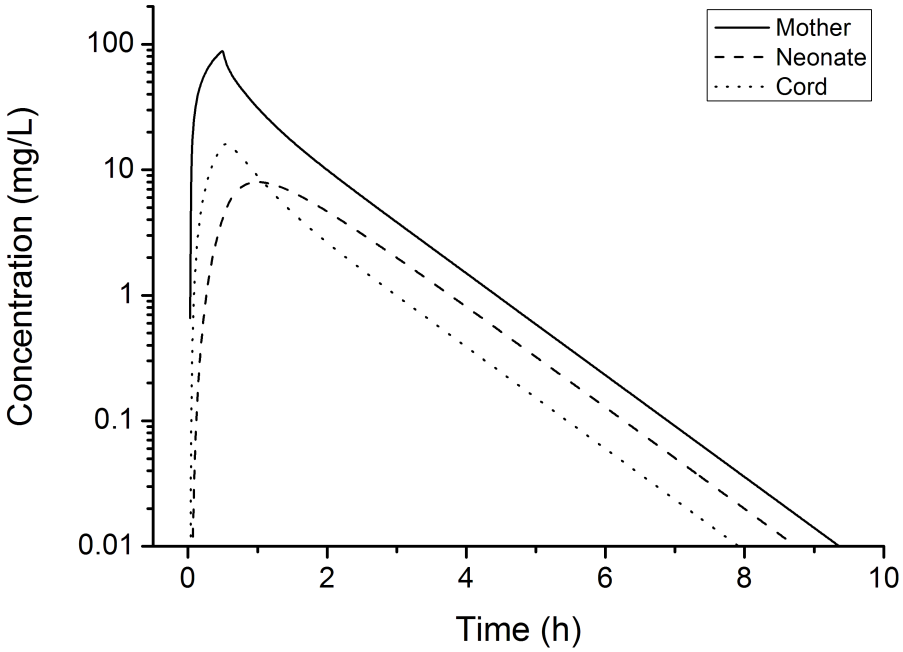

Figure 4: *Simulated concentration-time profiles for the mother, umbilical cord and neonate.*
Concentration-time profile of amoxicillin in maternal, umbilical cord and neonatal serum simulated after a single dose of 2 gram amoxicillin infused over 30 minutes. The simulations were performed with PK parameter estimates based on the final 5-compartment model and carried out for 12 hours after a single antibiotic dose. (See color inlay for a full color version of this figure.)

mass index, only gestational age was incorporated in the final model. The effect of other potential covariates was also assessed. This resulted in maximum decreases of OFV of –0.5 for body weight, -0.3 for the blood pressure, and -0.2 for pulse. All model with the temperature incorporated resulted in running-errors. The following equation represents the covariate model in the final model including the gestational age as covariate for the volume of distribution.

$$V_1 = \theta_2 \cdot (1 + \theta_{12} \cdot (GA - 36.8)) \cdot \exp(\eta_2)$$

In which V_1 is the volume of distribution of the central compartment, θ_2 is the estimate for V_1, θ_{12} is the estimate for the effect of gestational age and GA is the gestational age centered by its median value in the study population (36.8 weeks).

η_2 estimates the inter-individual variability on V_1. An increase in V_1 of 4.2% per week was found and incorporated into the model. The scatter plots of the observed concentrations versus model-predicted concentrations were randomly distributed, illustrating the unbiased model fit for maternal, umbilical cord and neonatal serum concentrations (figure 3). Table 2 shows the estimated values for the pharmacokinetic parameters of the final model. The volumes of distribution of the venous umbilical cord and the neonate were calculated (3.4 L for the umbilical cord and 11.9 L for the neonate).

The bootstrap validation of the model of the entire population was performed with 100 runs. The bootstrap validation was successful for 91 runs. The mean parameter estimates of the runs obtained from the bootstrap analysis deviated 1 to 36% from the predicted values from the NONMEM PK analysis, indicating that the accuracy of the final model is good.

The parameter estimates obtained by the population modeling analysis were used to simulate concentration-time profiles of amoxicillin in maternal, venous umbilical cord and neonatal serum after a single dose of 2 gram amoxicillin. Figure 4 shows the obtained concentration-time profiles for the initial dose of 2 g amoxicillin infused to the mother over 30 minutes. Maternal peak concentration was reached at the end of the antibiotic infusion at 30 minutes (88.7 mg/L). Peak concentration in venous umbilical cord serum was lower and delayed compared to the maternal peak concentration. Peak concentration in venous umbilical cord serum was 16.0 mg/L (18% of the maternal peak concentration) and was reached 3.3 minutes after the maternal peak concentration.

The sparse neonatal concentrations were analyzed simultaneously with the maternal and umbilical cord serum concentrations. The restricted blood supply to the extremities directly after birth and the need of an informed consent of both parents, were the main reasons for the limited number of samples from the neonates. Peak-concentration after the 2 gram infusion to the mother in neonatal serum was 8.0 mg/L (compared to 16.0 mg/L in venous umbilical cord serum). Similar concentrations were reached 1.1 h after the start of the infusion and afterwards the neonatal serum concentrations exceeded venous umbilical cord serum concentrations.

An intrapartum dose of 2 gram amoxicillin is commonly used to prevent neonatal GBS disease by achieving bactericidal concentrations in the fetus for a sufficient amount of time. According to the clinical breakpoints as determined by the EUCAST, for GBS, concentrations of at least 0.25 mg/L should be reached 9. The simulations show that the concentration of amoxicillin after a single 2 gram dose in the maternal, the venous umbilical cord and the neonatal serum, exceeds the minimally inhibitory concentration for more than 90% of the dosing interval.

Discussion

This collection of data from the mother, umbilical cord and neonate, was a unique opportunity to develop a 5-compartment model to describe the overall concentration versus time profile in maternal plasma, umbilical cord and neonatal plasma. Peak- concentrations in umbilical cord and neonatal serum were lower and delayed compared to the maternal peak-concentration. Approximately 1 hour after the start of the antibiotic administration the neonatal concentration reached its highest level, and thereafter exceeded the concentrations in venous umbilical cord. In a first approximation, simulation of a 2 gram infusion on basis of the developed pharmacokinetic model demonstrated that amoxicillin concentrations in maternal, venous umbilical cord and neonatal serum exceeded the minimal inhibitory concentration for >90% of the dosing interval.

Studies on the pharmacokinetics in umbilical cord serum are scarce. For ampicillin, an antibiotic closely related to amoxicillin, two studies have been performed in women during elective caesarean section[1,2]. Due to differences in study design direct comparison to our study is not possible. Because physical changes during labor might influence the transplacental blood flow, the transfer of amoxicillin across the placental barrier might be different during vaginal delivery. Furthermore, data in both studies were not analyzed using a population pharmacokinetic approach. Nevertheless, results of both studies are in line with our results. The ampicillin concentrations in cord serum exceeded the MIC for GBS during the study periods of 7-71 minutes[1] and 32- 343 minutes[2] after the start of the antibiotic infusion. Colombo et al.[2] focused on the ratio of the concentration in cord serum and maternal serum. They reported that initial cord serum concentrations were lower than maternal concentrations, but after approximately 80 minutes the concentrations in umbilical cord serum exceeded the maternal serum concentrations. In our study the venous cord concentrations did not exceed the maternal concentrations.

Both patients receiving amoxicillin and co-amoxiclav were included in the study. In the literature it has been described that the addition of clavulanic acid does not influence the pharmacokinetics of amoxicillin after intravenous administration[10-12]. Our data confirmed this finding (unpublished observations). Therefore, the pharmacokinetics of amoxicillin in these patients was assumed to be similar and all patients were analyzed simultaneously.

In the final analysis, the arterial umbilical cord and neonatal concentrations were considered similar. Several differences exist between these concentrations. Before clamping the umbilical cord antibiotics are eliminated from the fetus by transplacental transport to the mother and by fetal renal excretion. After cord clamping elimination of amoxicillin from the neonate takes place only by neonatal renal excretion. Because elimination from the neonate to the mother was included

in the model, the model predicted neonatal concentrations might be lower compared to the observed concentrations. Furthermore, many physiological changes occur immediately after birth in the neonate. These changes might influence the PK in the neonate. However, since all concentrations taken from the neonate and the arterial umbilical cord are distributed randomly around the line of identity, these differences do not seem to have a major influence on the PK within the first hours after birth

Blood samples in venous umbilical cord serum are sometimes used as a representative for concentrations in fetal serum. In the simulated concentration-time profiles the neonatal serum concentration is slightly higher than the concentrations in venous umbilical cord. This might be explained by a change in elimination route after birth. Before umbilical cord clamping the amoxicillin was eliminated both by the mother and fetus. After birth the amoxicillin is eliminated only by the neonate. Together with the decreased glomerular filtration rate in neonates, this results in a slower elimination rate and higher serum concentrations. This indicates that venous umbilical cord amoxicillin concentrations will not overestimate the concentrations in fetal serum.

In our study the simulated concentration-time profiles in maternal, venous umbilical cord and neonatal serum are used to describe the PK in relation to each other. In a first approximation, an amoxicillin infusion of 2 gram seems to be adequate to prevent neonatal GBS disease in the average patient. But to evaluate the efficacy of the dosing regimen as recommended by the CDC[8] for all pregnant women and to investigate possible improvement of the dosing regimen, further studies are needed. Such studies should take into account the inter-individual variability, correlation between the PK parameter estimates and knowledge of the concentration-effect relationship.

Acknowledgments

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Chapter 8

Evaluation of dosing regimen on amoxicillin exposure in pregnant women with preterm premature rupture of the membranes using Monte Carlo Simulation.

Anouk E. Muller, Lia (C.) Liefaard, P. Joep Dörr, Paul M. Oostvogel, Joost de Jongh, Eric A.P. Steegers, Rob A. Voskuyl, Meindert Danhof, Johan W. Mouton

Abstract

Objective: To determine whether the probability of target attainment differs significantly between commonly used amoxicillin dosing regimens and whether inaccuracies in antibiotic administration influence the probability of target attainment.

Study Design: Population pharmacokinetic parameter estimates and their inter-individual variability in patients with premature rupture of the membranes were obtained from a previous investigation. Monte Carlo Simulations were then used to determine the influence of differences in the dosing of amoxicillin on the percentage of time that serum concentrations of the unbound drug remained above the Minimum Inhibitory Concentration (%*f*T>MIC).

Results: When administering 1 gram as a bolus injection, %*f*T>MIC was approximately 4% lower than after infusion over 15 minutes. Simulating the administration of 1 gram amoxicillin bolus injections every 6 hours, the %*f*T>MIC at steady state for the average population is 92%, for a value of 0.25 mg/L as MIC of group B streptococcus (GBS). When the lower 99% confidence interval (CI) was taken into account, this percentage decreased to 46%. After decreasing the dosing interval to 4 hours, the %*f*T>MIC for the average population and the lower 99% CI were 99% and 70%, respectively.

Conclusion: Minor differences in the probability of achieving effective concentrations were detected between the different dosing regimens of amoxicillin. A dosing schedule of 1 gram bolus injections every 6 hours is adequate to prevent GBS infections and minor inaccuracies in the administration are not influencing its predicted efficacy. An initial dose of 2 gram is has no added value.

Introduction

During labor, especially after rupture of the membranes, pregnant women and their fetuses are at risk for ascending infections from the vagina. An important part of these infections is caused by group B streptococcus (GBS, *Streptococcus agalactiae*). Up to 35% of pregnant women are colonized with GBS in the rectovaginal tract[1,2]. To protect neonates of these mothers from infection, prevention strategies have been implemented[3,4]. Antibiotics are used in the management of pregnant patients to eliminate GBS from the site of infection. Proper dosing of antibiotics is essential to prevent both mother and fetus from infection.

Although several dosing regimen recommendations exist and are currently used, the rationale behind these regimens is not always clear. Specifically, limited information on the pharmacokinetics of antibiotics in pregnancy in general and in the peripartal period in particular, is available. In addition, at the time these regimens were designed less information was available with respect to the pharmacokinetic/pharmacodynamic properties of the drugs used. This may lead, or may have lead, to therapeutic failure. Cases of neonatal GBS infection after maternal antibiotic therapy have been described[5,6]. In the literature different dosing regimens for the prevention of GBS infection are recommended. Guidelines to prevent neonatal GBS disease issued by the Centers for Disease Control and Prevention (CDC) recommend the use of an initial dose of 2 gram ampicillin and subsequent doses of 1 gram every 4 hours[4]. The Cochrane Library on the other hand describes a dosing regimen of ampicillin 1 gram intravenously every 6 hours as the usual regimen[7]. Because the consequences of changes in antibiotic dosing are unknown, it is not possible to study different regimens in pregnant women. Computer-simulations using data of the prescribed regimens are an accepted alternative, particularly when detailed information on the pharmacokinetics and the inherent inter-individual variation is available.

Monte Carlo Simulation (MCS) is used to evaluate the probability of achieving therapeutic concentrations of different dosing schedules. MCS is performed using pharmacokinetic parameters, data on the concentration-effect relationship and on the inter-individual variability[8-13]. The relationship between pharmacokinetic properties, the susceptibility of the microorganism (as reflected in the Minimum Inhibitory Concentration, MIC) and clinical effects is increasingly well understood. Specifically, the efficacy of the penicillins such as ampicillin and amoxicillin, has been shown to be primarily correlated to the percentage of time that serum concentrations of the unbound drug remain above the MIC (%*f*T>MIC)[14-16]. In general, the therapeutic goal to cure infections caused by Gram-positives is a %*f*T>MIC of at least 40%, which corresponds to an in vivo static effect in animal studies. The pharmacokinetic behavior of drugs differs for each individual. The recommended dosing schedule should be adequate in preventing infections for the

entire population. Therefore, inter-individual variability is a determining factor in the prediction of the outcome of therapy in individual patients. To predict the probability of success of treatment, the %*f*T>MIC should be at least 40% for each individual within the population.

Outside the US, intravenous amoxicillin is often used in the prevention of neonatal GBS disease. Amoxicillin dosing regimens have been derived from studies using ampicillin, a beta-lactam antibiotic closely related to amoxicillin[17, 18]. Differences in dosing regimens, as well as inaccuracies in the administration of the antibiotic might influence the %*f*T>MIC and the associated efficacy in preventing GBS infections. The purpose of this study was therefore to perform MCS for amoxicillin concentrations in pregnant women to assess whether the probability of target attainment (PTA) differed significantly between the recommended dosing regimens and to examine the influence of inaccurate antibiotic administration on PTA. To this end, we used the population pharmacokinetic model for amoxicillin in pregnant women with preterm premature rupture of the membranes (PPROM) as previously described[19].

Materials and Methods

Dosing schedules

The different dosing regimens used for the MCS are shown in table 1.

Simulated dosing regimen			
	amount	**infusion**	**interval**
Single dose			
	1 gram	15 min	4 h
	1 gram	Bolus	4 h
	1 gram	Bolus	6 h
	2 gram	30 min	4 h
Steady state			
	1 gram	Bolus	4 h
	1 gram	Bolus	6 h
Inaccuracies			
	1 gram	Bolus	8 h
	1 gram	Bolus	12 h
	1 gram	30 min	4 h
	1 gram	Rate 66.7 mg/min	-

Table 1: *Simulated dosing regimens.*

Study population and pharmacokinetic modeling

The population pharmacokinetic model of the amoxicillin serum concentrations in pregnant women with PPROM has been described previously[19]. The amoxicillin was administered intravenously. The initial dose of 2 gram was infused over 30 minutes and the subsequent 1 gram doses were infused over 15 minutes. Population pharmacokinetic parameter estimates were based on a population analysis of plasma concentrations in 17 patients using NONMEM. A total of 416 blood samples was obtained and used in the modeling procedure. A 3-compartment open model best described the concentration-time curves. Inter-individual variability was very small and largely explained by variations in the parameters clearance and volume of distribution of one of the peripheral compartments. The main demographic characteristics and pharmacokinetic estimates are summarized in table 2.

	Demographic characteristics			Structural model parameters		
	mean	SD	range		mean	SE
Maternal age (y)	29.42	4.64	19.6-35.1	CL (L/h)	22.8	1.03
Gestational age (wk)	35.1	1.63	29.4-36.9	V_1 (L)	5.59	0.826
Body mass index (kg/m^2)	29.1	3.87	21.5-35	V_2 (L)	7.43	1.06
Weight (kg)	80.9	12.03	56.2-98.9	V_3 (L)	8.61	0.768
Leucocytes x10^{9}L	11.8	4.43	6-25.9	Q (L/h)	60	18.5
Creatinin µmol/L	44.4	10.11	37-74	Q_2 (L/h)	7.72	1.72

Table 2: *Main demographic characteristics and pharmacokinetic estimates of the 17 pregnant women with PPROM.*
CL: Clearance, V_1: volume of distribution of the central compartment, V_2: volume of distribution of the first peripheral compartment, V_3: volume of distribution of the second peripheral compartment, Q: intercompartmental clearance between V_1 and V_2, Q_2: intercompartmental clearance between V_1 and V_3. More details can be found in Muller et al.[19].

Monte Carlo Simulation

The estimates of the pharmacokinetic parameters and measures of dispersion were used to simulate various dosing regimens and obtain %*f*T>MIC as a function of MIC[13]. An amoxicillin protein binding of 22% was used in the simulations. MCS was performed using the MICLAB version 2.36 program (Medimatics, Maastricht, the Netherlands) simulating 10.000 subjects for each regimen. The program allows inclusion of the covariance matrix (or correlation matrix) of the parameter

estimates used in the simulations. The output consisted of a probability distribution, a cumulative probability distribution, and specific confidence intervals over user defined MIC and %*f*T>MIC ranges. MIC breakpoint of GBS for amoxicillin of 0.25 mg/L was used according to the susceptibility breakpoints determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[20].

Results

The values for %*f*T>0.25 mg/L for the dosing regimens obtained from the MCS are shown in table 3. After an initial bolus injection of 1 gram the %*f*T>MIC was approximately 4% lower compared to the value after a 15 minutes infusion. The use of a loading dose of 2 gram administered over 30 minutes increased the %*f*T>MIC for the population including the 99% CI with 19%, but both values remained above the targeted value of 40%.

The most frequently used dosing intervals (4 and 6 hours) were also simulated in a steady state situation. Figure 1 shows the %*f*T>MIC for these 2 dosing intervals. Simulating the administration of 1 gram amoxicillin every 6 hours as bolus injection the %*f*T>MIC in maternal serum for the average population was 92%, given the MIC of GBS. When the lower 99% CI was taken into account, this percentage decreased to 46%. For the dosing interval of 4 hours as recommended

Simulated dosing regimen				%*f*T>0.25 mg/L		
	amount	**infusion**	**interval**	**average**	**95% CI**	**99% CI**
Single dose						
	1 gram	15 min	4 h	99.3	88.1	73.2
	1 gram	Bolus	4 h	99.1	84.5	69.4
	1 gram	Bolus	6 h	91.4	56.8	47.9
	2 gram	30 min	4 h	99.9	99.6	88.8
Steady state						
	1 gram	Bolus	4 h	99.1	84.2	69.7
	1 gram	Bolus	6 h	91.5	55.7	46.3
Inaccuracies						
	1 gram	Bolus	8 h	77.8	42.2	34.9
	1 gram	Bolus	12 h	53.3	28.2	23.3
	1 gram	30 min	4 h	99.4	90.1	74.6
	1 gram	Rate 66.7 mg/min	-	-	-	-

Table 3: *Simulated dosing regimens with the %fT>0.25 mg/L. The MIC of amoxicillin for GBS was determined by the EUCAST.*

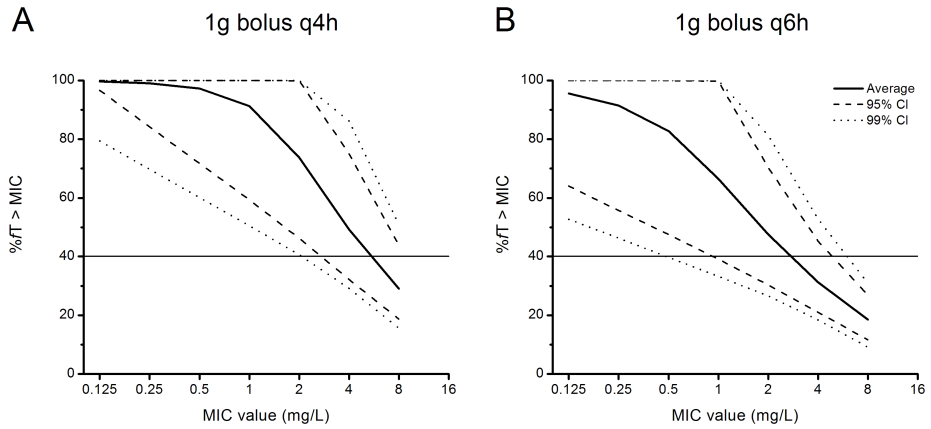

Figure 1: *Percent of time the unbound fraction of amoxicillin remained above the MIC (%fT>MIC) as a function of the MIC for two dosing intervals, 4 hours (figure 1a) and 6 hours (figure 1b), in pregnant women with PPROM in steady state situation.*

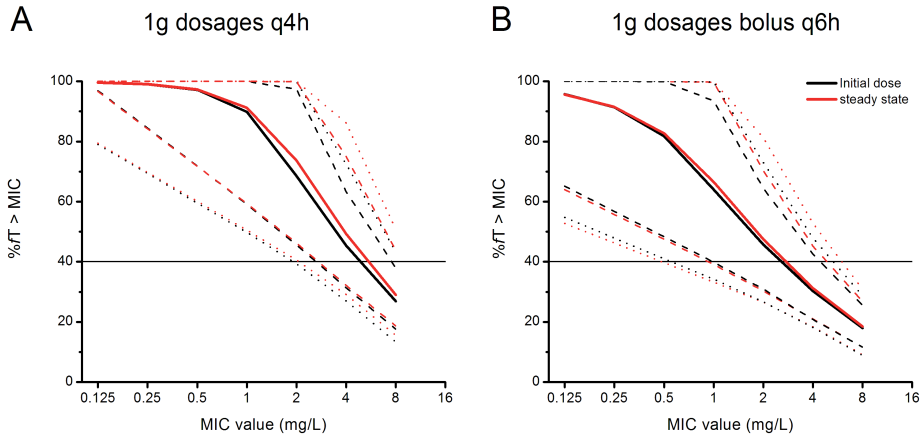

Figure 2: *Percent of time the unbound fraction of amoxicillin remained above the MIC (%fT>MIC) as a function of the MIC for two dosing intervals, 4 hours (figure 2a) and 6 hours (figure 2b), in pregnant women with PPROM after the inital dose (black lines) and in steady state situation (red lines). (See color inlay for a full color version of this figure.)*

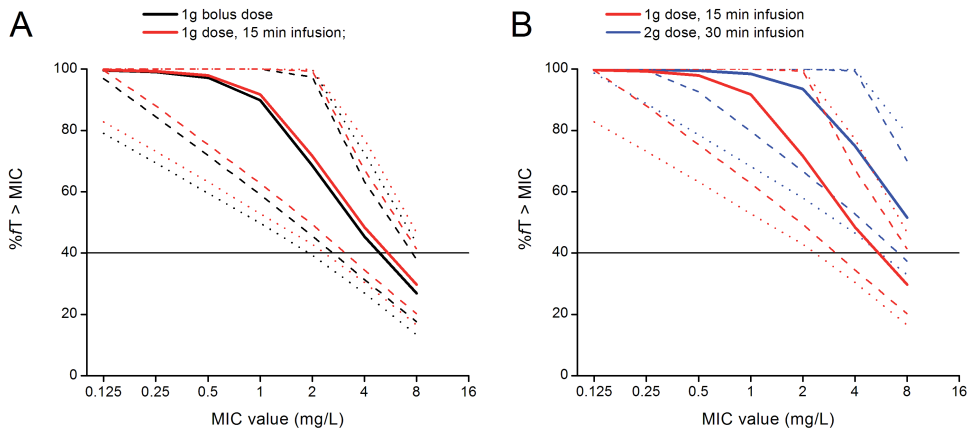

Figure 3: *Percent of time the unbound fraction of amoxicillin remained above the MIC (%fT>MIC) as a function of the MIC for three different initial doses for a 4 hours dosing interval in pregnant women with PPROM. In figure 3A the %fT>MIC for a dose of 1 gram administered as bolus (black lines) and the 1 gram dose administered over 15 minutes (red lines) are shown. In figure 3B the %fT>MIC for the 1 gram dose administered over 15 minutes (red lines) and the 2 gram dose administered over 30 minutes (blue lines) are shown. The solid lines are the values for the average pregnant women; the interrupted lines represent the 95% confidence intervals and the dotted lines the 99% confidence intervals. (See color inlay for a full color version of this figure.)*

by the CDC the %*f*T>MIC for the average population and the lower 99% CI were 99% and 70% at steady state, respectively. Therefore the probability of target attainment of amoxicillin administered every 6 hours would probably be lower than when administered every 4 hours, but the %*f*T>MIC for all patients exceeded the target percentage of 40%. As shown in figure 2, there are minor differences in the shape of the curves for initial dose and for doses administered at steady state at dosing intervals of 6 hours and 4 hours. In figure 3, the %*f*T>MIC for the three different initial doses is shown (1 gram bolus, 1 gram infusion over 15 minutes and 2 gram infused in 30 minutes). This indicates that the use of a loading dose of 2 gram amoxicillin is not beneficial to achieve adequate concentration-time profiles in maternal serum to prevent GBS disease.

During daily patient care, the ideal infusion time is sometimes hampered by small accidents, overlooks or other misfortunes, resulting in possibly inadequate concentration profiles. We simulated some of these possible adverse regimens. In the first simulation the interval was extended to 8 hours and 12 hours respectively, predictably resulting in decreased %*f*T>MICs. Given the MIC of GBS, the %*f*T>MIC for the average population is 78% for an interval of 8 hours and 53% with an

interval of 12 hours. However, when the 99% CI was included in the analysis of a dosing interval of 8 hours and 12 hours the %*f*T>MIC decreased to values below the 40%. Another problem frequently occurring is obstruction during infusion. When an obstruction in the infusion system results in a slower administration the %*f*T>MIC is increased. When amoxicillin was administered with a rate of 66.7 mg/min (1 gram over 15 minutes) with a dosing interval of 4 hours, the %*f*T>MIC was >50% for the population with the 95% CI included when the infusion is stopped after 2 minutes.

Discussion

We determined the effect of the use of different dosing regimens on the probability of achieving therapeutic concentrations in the entire group of pregnant women with PPROM. Monte Carlo simulation using pharmacokinetic estimates and a three-compartment model showed that both the dosing regimen as recommended by the CDC[4] and the regimen mentioned in the Cochrane Library[7] result in adequate concentration-time profiles in maternal serum. For both regimens accidentally missing a single dose results in a %*f*T>MIC above the threshold for efficacy for the majority of the population. Inaccuracies in the rate of the infusion of amoxicillin did not have clinically relevant changes on the PTA, primarily because the MIC of GBS is relatively low.

The CDC recommends an initial dose of 2 gram of ampicillin[4]. For the average population the %*f*T>MIC after such a loading dose for amoxicillin as well as after a bolus infection of 1gram is >99%. Taking into account the 99% CI this difference was 19%, but the values were above the target of 40%. Adequate concentrations were reached almost immediately after the start of the administration. Therefore the use of a 2 gram loading dose does not seem to be beneficial for reaching adequate maternal concentration-time profiles.

The rate of the administration of 1 gram amoxicillin does not have major influences on the %*f*T>MIC of the concentration-time profiles. The %*f*T>MIC after a bolus injection is only slightly lower compared to the standard infusion over 15 minutes. It is only when amoxicillin infusions are extended to hours, that major differences will result (results not shown). This is in line with the half life of amoxicillin.

An obstruction in the infusion system occurs regularly and in clinical practice finally the total amount of antibiotic is administered. Using MCS, it is not possible to simulate this particular situation. The best alternative is to simulate an infusion 1 gram with a constant rate of infusion and stop the infusion prematurily. In this way, a minimal infusion time was determined needed to reach the target %*f*T>MIC of 40%. This target was reached for the majority of the population after

2 minutes of infusion, but in clinical practice the infusion will only be temporarily blocked resulting in much higher values for %*f*T>MIC.

Studies showing a clear relationship between exposure to amoxicillin and its efficacy in preventing GBS infections in pregnant women are not available. To our opinion it is reasonable that in this patient group the exposure should correspond to exposures that correlate to a 1 to 2 log drop of colony forming units in various models, and thus be bactericidal rather than bacteriostatic. Thus, for amoxicillin the %*f*T>MIC should therefore be at least 40%.

Only the unbound fraction of the total drug concentration is active. Therefore, the percentage of protein binding is implemented in the MCS. Values for protein binding of amoxicillin vary between 18-20%[21,22]. Protein binding might even decrease in pregnant women[23]. It is likely that the protein binding of 22%, as used in our analysis, underestimates the active fraction of amoxicillin in pregnant women. The use of 22% is therefore a conservative estimate. This may lead to slightly underestimated %*f*T>MICs resulting from the simulations.

When prevention of GBS infection fails and the fetus gets infected, adequate fetal serum levels and probably amniotic fluid levels are required as well. Amoxicillin reaches the fetus after transplacental transfer from the mother. Consequently, adequate maternal serum levels are a prerequisite for adequate levels in fetal serum. Concentrations in fetal serum are lower compared to concentrations in maternal serum; therefore these simulations can not guarantee that these dosing regimens are adequate for the prevention of GBS infection in the fetus.

In conclusion, we present MCS using a three-compartment model. Both the dosing regimen as recommended by the CDC and as mentioned in the Cochrane Library will result in adequate concentrations in maternal serum. A two gram loading dose does not seem to be beneficial and the 1 gram doses can safely be administered by bolus injection increasing the comfort of the patient and facilitating prophylaxis. For the majority of the population considerable inaccuracies in the infusion of amoxicillin will not interfere with its predicted efficacy in preventing infections with GBS in the mother. A dosing regimen of bolus injections of 1 gram every 6 hours was predicted to be adequate for the prevention GBS infection in pregnant patients.

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Chapter 9

Aberrant amoxicillin pharmacokinetics in a pregnant patient with severe vomiting: a case-report.

Anouk E. Muller, Johan W. Mouton, Paul M. Oostvogel, P. Joep Dörr, Eric A.P. Steegers, Meindert Danhof, Rob A. Voskuyl

Pregnant women included in earlier studies were all relatively healthy[1,2]. Contrary to expectations these studies did not reveal large differences in pharmacokinetics between pregnant and non-pregnant individuals. In fact, the pharmacokinetics of amoxicillin in these patients were surprisingly similar compared to non-pregnant individuals. However, in rare instances the results may be dramatically different. Here we describe the case of a patient whose pregnancy was complicated by several factors starting from the 23th week. She was treated with amoxicillin because she had preterm premature rupture of the membranes (PPROM). After analysis of the amoxicillin concentrations it turned out that the pharmacokinetics were strikingly different in this patient compared to the pharmacokinetics in patients with PPROM as described earlier[1]. Her case is described here to demonstrate how unusual physiological and pathological conditions, and perhaps the medical interventions as well, can indeed affect the pharmacokinetics of a drug.

Case

A 30-year-old primigravid woman presented to our hospital with a monochoreal diamniotic twin-pregnancy.
Her medical history only reported a hernia inguinalis operated in 1982. Her twin-sister had been diagnosed with multiple sclerosis.

From the 23th week of gestation she was complaining of pyrosis. At a gestational age of 25 weeks she was admitted to our hospital with unexplained nausea and vomiting. She was successfully treated with aluminiumoxide/magnesiumhydroxide (Antagel®), an antacid. Unfortunately, her stay in hospital was complicated by a deep venous thrombosis of her left leg. Therefore, she was treated with nadroparin (Fraxodi®). Nine days after she had been admitted, she left the hospital with minor discomfort of the stomach, but without vomiting.

At a gestational age of 29 weeks, she presented with waxing and waning pain in her back, referring to her abdomen and she had some vaginal fluid loss observed. She was complaining of nausea since a couple of hours. It could not be determined whether the pain was caused by uterine contractions. Uterine contractions were not felt by external palpation and external electronic fetal monitoring did not register regular contractions. During speculum examination some clear fluid was seen and the cervix appeared to be opened for 1 cm. Although the fern-test was negative, the fluid seen in the vagina was suspected for amniotic fluid. Upon admission she had a pulse-rate of 82 beats per minute and a temperature of 36.8 °C. Her hematological parameters were as follows: hemoglobin 7.3 mmol/L, hematocrit 0.37 L/L, platelets 353 x 10^9/L, leucocytes 10.6 x 10^9/L, C-reactive protein 7 mg/L.

Based on the history of uterine contractions, signs of cervical dilatation and

ruptured membranes, she was diagnosed with a threatening preterm delivery. It was decided to treat her with tocolytic drugs and antibiotics. Amoxicillin was prescribed for the ruptured membranes. She agreed to participate in our pharmacokinetic study.

Initially, tocolytic therapy with nifedipine (Adalat®) was started. Nifedipine was administered orally and she started to vomit frequently. The capsules were thrown out. The intensity of the vomiting increased to a loss of 100-400 ml vomit once every 3 to 4 minutes. The vomit was dark brown and contained haemoglobin. Her heart-rate had increased. According to the study protocol two intravenous catheters were placed. One at the left hand for administration of the amoxicillin and a second catheter at her right arm for sampling. A 2 gram dose of amoxicillin in 30 cc 0.9% NaCL was administered intravenously. The haematological parameters were similar to those upon admission. The liver functions were slightly enhanced (AF 273 U/L, ASAT 56 U/L, ALAT 77 U/L, γGT 39 U/L) and the renal function was normal (ureum 3.2 mmol/L, creatinin 59 μmol/L, uric acid 0.35 mmol/L). After the first antibiotic dose, it was very difficult to draw blood from the sampling catheter during the first 4 blood samples at respectively 2, 16, 27 and 33 minutes after the start of the infusion. It was offered to the patient to stop the study, but she insisted on continuation. Subsequent blood samples could be obtained without problems out of the sampling line. Because she was still complaining of pain in her back, it was decided to start tocolytic therapy with intravenously administered ritodrin, a beta-2 agonist. Ritodrin was started at a low dose (200 μg/min) approximately one hour after the start of the amoxicillin. Her heart-rate increased up to 120 beats per minute, which was attributed to a side effect of the ritodrin. She had no fever.

Although she was treated with aluminiumoxide/magnesiumhydroxide (Antagel®) and 40 mg pantoprazole (Pantozol®) intravenously in the first hours after the amoxicillin infusion, she continued vomiting. To prevent the fetuses from respiratory distress syndrome she received an intramuscular dose of 11.4 mg celestone chronodose. At 3.5 hours after the first amoxicillin dose she received a rectal dose of metoclopramide (Primperan®) and fluid suppletion was increased. Subsequently she stopped vomiting and fell asleep. She reported some decrease in the pain in her back.

The second dose of 1 gram amoxicillin was administered with an interval of 4 hours after the first dose using the same intravenous catheter located on her left hand. At that time she was sleeping. Serial blood samples were easily obtained during the next four hours.

At a gestational age of 30 weeks and 4 days the patient delivered. Her two daughters were admitted to the paediatric department because of their prematuritas. They did not present signs of infection. The placenta was examined by the pathologist. In the diamniotic monochorionic twin placenta were no signs of infection.

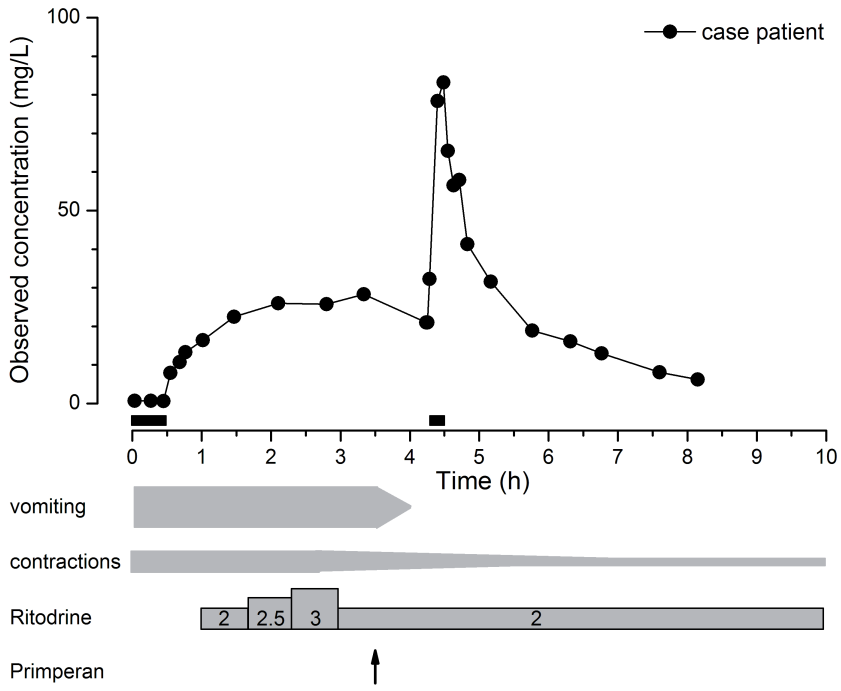

Figure 1*: Concentration-time profile of the case-patient. Important medical information is shown as well. Ritodrine 2 equals 200 µg/min.*

Blood samples were placed immediately on ice and processed within 1 hour after collection. The samples were centrifuged at 1200 g for approximately 10 minutes. The supernatants were transferred into plastic storage tubes and frozen at -70° C until analysis. To determine the amoxicillin concentrations a validated high-pressure liquid chromatography was performed as described previously[1].

Concentration-time profiles of amoxicillin in this patient, and timing of medication, vomiting and uterine contractions are shown in figure 1. The profile after the first administration was different from the profile after the second administration. After the first dose, a low antibiotic concentration of 0.7 mg/L was measured in blood drawn from the opposite arm. Afterwards the concentration increased and the peak concentration was reached approximately 200 minutes after the end of the antibiotic infusion. Peak-concentration after the second dose was reached at the end of the infusion. The area under the curve (AUC) was calculated using the trapezoid rule for the first, 2 gram, amoxicillin administration in the case-patient, as well as for the concentration-time profiles simulated using Berkely Madonna for patients with PPROM after a 2 gram and 1 gram infusion[1]. For the 2 gram infusion

the $AUC_{0\text{-}4.23}$ in this patient was 85.5 h·mg/L and 78.9 h·mg/L for the mean PPROM patient. $AUC_{0\text{-}4.23}$ for the mean PPROM patient after a 1 gram infusion was 37.0 h·mg/L.

Discussion

We described the concentration-time profile of amoxicillin in a patient with threatening preterm delivery. Her treatment was complicated by unexplained severe vomiting during the first amoxicillin infusion. At the time the second amoxicillin dose was infused the patient stopped vomiting and the frequency of the waxing and waning abdominal pain slightly decreased. The concentration-time profile was determined during the first and second antibiotic doses of respectively 2 and 1 gram amoxicillin (figure 1). The profile measured during the second infusion was comparable to results from earlier studies[1]. Figure 2 shows the concentration-time profile of the case-patient and the concentration-time profile of a control-patient from our previous study. The control-patient was also a primigravid woman with a twin-pregnancy and PPROM.

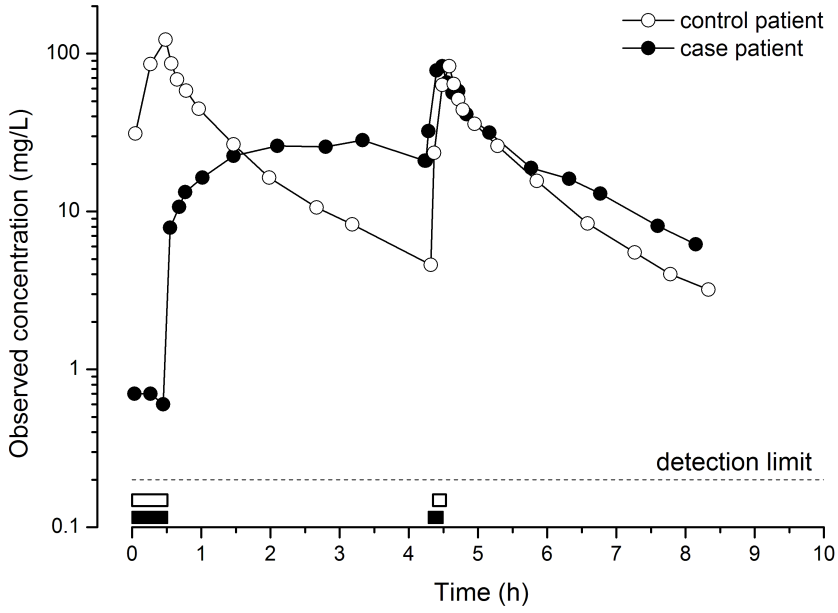

***Figure 2**: Concentration-time profiles of the case patient and control patient. The black and white bars indicate the amoxicillin infusion. The detection limit represent the amoxicillin detection as well as the quantification limit.*

From the shape of the profile after the first dose it appeared that the amoxicillin distributed differently through the body when compared to the second dose. This raised a number of questions. In the first place, was the aberrant profile after the first amoxicillin administration an artefact caused by technical difficulties during administration and did it represent the true amoxicillin concentrations at the site of sampling? Second, if it was not an artefact, how could the deviating pharmacokinetics be explained? Third, if it represented the true plasma concentrations of amoxicillin, did this have consequences for the purpose for which amoxicillin was given, i.e. prevention of infection in the foetus?

Firstly, possible explanations due to deviations in the practical execution of the study were considered. Both antibiotic doses were administered using the same catheter. Therefore, the difference between the concentration-time profiles obtained after the first and second antibiotic dose, can most likely not be explained by an accidentally subcutaneous administration of the first dose. Moreover, the antibiotics were administered using an infusion pump, which produces a warning sound when there is an obstruction in the system. The investigator was continuously present during the infusion and did not observe difficulties in the infusion. Furthermore, the AUC of the aberrant curve is not smaller compared to the AUC in healthy patients with PPROM. This indicates that the entire dose is absorbed. Normally, blood pressure is measured once every 15 minutes when a patient is treated with ritodrine. Due to the emotional and physical state of the patient her blood pressure was not measured. Therefore there is no possibility that the blood pressure cuff obstructed the blood flow in the arm.

An aberrant concentration-time profile might also be explained by an accidental exchange or faulty labelling of blood samples or by errors in the determination of the amoxicillin concentrations. The profile after the first dose was based on 12 blood samples. The concentration data of all samples produced a smooth curve, therefore our aberrant observation can not be explained by outliers. The amoxicillin concentrations in all samples were determined using the same HPLC-method and controls were included in every run. Consequently, it is unlikely that this profile is aberrant due to errors in the HPLC-method. Therefore, it is concluded that the concentrations as shown in figure 1 appear to represent the true concentrations at the sampling site.

To explain the aberrant concentration-time profile, physiological changes that may influence the pharmacokinetics of amoxicillin were considered. For various drugs it is known that the pharmacokinetics is influenced by the state of pregnancy and /or being in labour. As an example, the pharmacokinetics of ampicillin, an antibiotic structurally closely related to amoxicillin, has an decreased half-life in pregnant women according to several studies[3-6]. Other studies described an increased half-life of ampicillin, but exclusively in women during labor compared to non-pregnant individuals, not in pregnant women before the onset of labor[7,8]. In

our patient the aberrant pharmacokinetic profile was demonstrated only after the first (2 gram) dose. After the second (1 gram) dose the profile was comparable to the profiles described for pregnant women with PPROM[1]. The state of pregnancy and labor did not change between the two doses and it has been shown previously that shape of the concentration-time curve was not changed by pregnancy or labor[2]. Therefore, this aberrant profile cannot be explained by pregnancy or labor.

Additional factors might also influence the pharmacokinetics. This patient had a lot of psychological stress, was vomiting seriously and various drugs were administered in the period when the amoxicillin was infused. Psychological stress activates the sympathic nervous system, thereby inducing a cascade of physiological reactions, finally resulting in strengthening of the contractility of the heart and peripheral (arterial) vasoconstriction. Vasoconstriction limits the blood flow to the limbs as well as increases the critical closing pressure. An increased critical closing pressure facilitates the occlusion of the blood flow in restricted areas.

The possible physiological changes as a result of the serious vomiting are more complex. An enormous effort for the body is required to vomit frequently. The blood flow to the muscles involved in vomiting, mainly the respiratory and abdominal muscles, might change the distribution of blood through the body and restrict blood flow to less vital areas such as the arms. The fluid loss resulting from the vomiting might cause a (minor) hypovolemia. Clinically, an increased heart-rate will be seen. In our patient the heart-rate was increased but this was attributed to a possible intrauterine infection, stress and a side effect of one of the administered drugs (ritodrine). Initially, no fluid replacement was given. After large amounts of fluid loss, the hematocrit values are likely to increase. In patients with an increased hematocrit, the blood viscosity is increased because of cell deformation, which will result in a reduced blood flow. However, The hematocrit value in our patient was similar 120 min and 20 min before the start of the amoxicillin. Both due to the short time interval between the two measurements and to a limited blood flow in the arm, it is reasonable to that the hematocrit remained similar. Thus, it is inferred that the main effect of vomiting was to redistribute blood flow over the body and to restrict flow in the arms

In the study period, several drugs were administered besides the amoxicillin. It is unlikely that the Antagel® or Pantozol® influenced the pharmacokinetics. However, ritodrine has a dilatating effect on the blood vessels. Because vasoconstriction in patients with hypovolemia occurs as compensation mechanism, ritodrine is contraindicated in those patients. Vasodilatation is these patients might result in venous stasis, thereby limiting the blood flow. In our patient ritodrine was administered 60 min after the start of the amoxicillin and in a low dose (200 μg/min). The dose was increased minimally (see figure 1), because of the increase in heart-rate. This increased might be a side effect of the ritodrine, but might also in part be explained by a reaction on vasodilatation caused by hypovolemia.

From the clinical point of view, it is important whether the deviation of this concentration-time profile from the average profile affects the efficacy of the amoxicillin in the prevention of both maternal and neonatal GBS infection. The efficacy of amoxicillin is determined by the time the concentration exceeds the minimum inhibitory concentration (T>MIC). In general a T>MIC for 40-50% of the dosing-interval is required for efficacy[9-11]. The MIC-value of amoxicillin for GBS as reported by the EUCAST is 0.25 mg/L[12]. The concentration in maternal serum of our patient exceeds the MIC-value for GBS within 2 minutes after the start of the infusion and stays above the MIC for the remaining time of the dosing-interval. This aberrant concentration-time profile in maternal serum is therefore unlikely to reduce the efficacy of the amoxicillin in preventing maternal GBS infection. However, the main goal of this antibiotic administration is preventing the fetus from infection. Since the transfer of amoxicillin over the placental barrier might be influenced by the peak-concentration in maternal serum, the efficacy in preventing the fetus from infection might be reduced. Unfortunately, concentrations in umbilical cord blood and fetal blood could not be obtained in this case and it is therefore unknown whether the amoxicillin in this patient was adequate during the first dosing-interval to prevent the fetus from GBS infection.

In 1972, Rowland et al also reported an aberrant pharmacokinetic profile[13]. In this case, a male volunteer became faint 10 minutes after he received an oral dose of 650 mg aspirin. He recovered soon afterwards and during this event the study was continued. From the moment he became faint, the absorption stopped and aspirin levels decreased for the next 20 minutes, where after the levels began to rise again. Fainting is the result of a decline in the blood flow to the brain. To compensate for this, vasoconstriction outside the heart and brain is likely to occur, resulting in a redistribution of the blood flow, favouring the flow to the brain. A decreased motility of, and a decreased circulation to, the gastrointestinal tract probably explain this aberrant curve. In our case, the blood flow is also likely to be redistributed, favouring, besides the brain and heart, the body components used in vomiting like the respiratory muscles, abdominal muscles and the gastrointestinal tract. Therefore the blood flow to the extremities will be minimized. In both cases redistribution of the blood flow is a possible underlying mechanism causing the aberrant PK profiles.

In conclusion, the concentration-time profile of amoxicillin after the first infusion in our patient deviated from the normal profile. It is not possible to explain the course of the profile with certainty, however we hypothised that several physiological changes had occurred that all influences the peripheral blood flow and thereby changing the distribution of the amoxicillin. The arm in which the amoxicillin was infused might act as depot for the amoxicillin. After the blood flow slowly normalises the amoxicillin is steadily released from the depot. The registration of this profile is unique because blood samples were taken frequently in an acute,

emotional and very stressful situation. It is not possible to perform pharmacokinetic studies of a group of patients in this condition. Therefore, individual cases are valuable and indicate that although the pharmacokinetics is quite well described in healthy volunteers and several groups of patients, unexpected differences in the pharmacokinetic profile can occur in (critically) ill patients.

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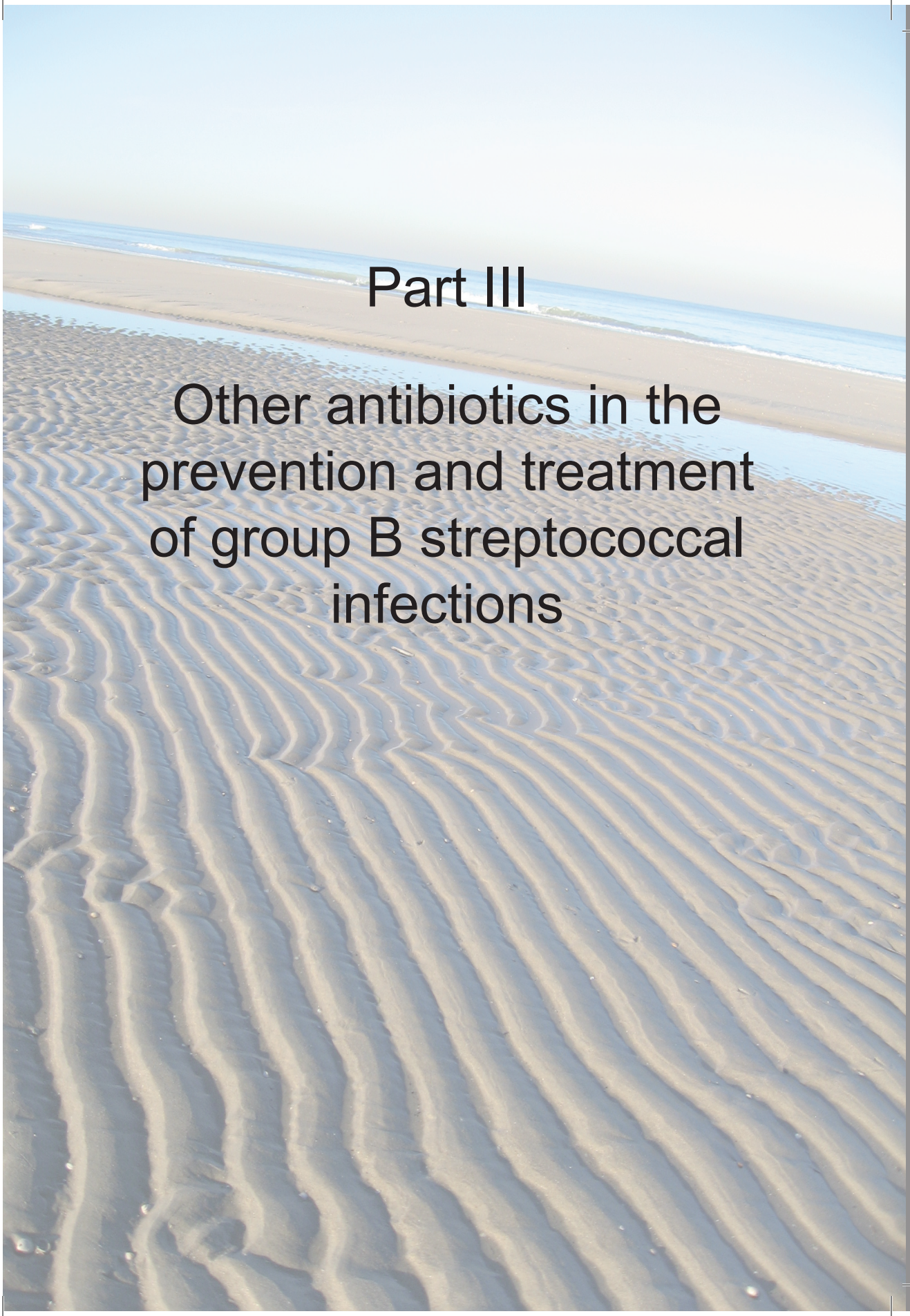

Part III

Other antibiotics in the prevention and treatment of group B streptococcal infections

Chapter 10

The pharmacokinetics of clindamycin in pregnant women in the peripartum period.

Anouk E. Muller, Johan W. Mouton, Paul M. Oostvogel, P. Joep Dörr, Rob A. Voskuyl, Joost de Jongh, Eric A.P. Steegers, Meindert Danhof

Abstract

Objective: This study was undertaken to describe the pharmacokinetics of intravenously administered clindamycin in pregnant women.
Study Design: Pregnant women were recruited who needed treatment with clindamycin in the prevention of neonatal group B streptococcal disease or of endocarditis (900 mg every 8 hours and 600 mg every 6 hours respectively). Following delivery, both arterial and venous umbilical cord blood samples were obtained. Clindamycin concentrations were determined with the use of high-pressure liquid chromatography. Nonlinear mixed-effects modeling was performed in NONMEM.
Results: The pharmacokinetics of 7 patients was best described by a three-compartment model. Clearance and volume of distribution at steady state were 10.0 L/h and 6.32 x 10^3 L, respectively. Using a 900 mg dosing regimen in the average pregnant women, the ratios of the area under the concentration time curves in maternal serum over the Minimum Inhibitory Concentration (MIC) were 64, 32, and 8 for assumed protein binding of 60%, 80% and 95% respectively. Concentrations in umbilical cord were lower compared to the maternal concentrations.
Conclusion: Concentration-time profiles in maternal serum are likely to be adequate for the average pregnant woman. In contrast the observed concentrations in arterial umbilical cord blood indicate that the current dosing regimen may not be adequate to prevent the neonate from group B streptococcal disease.

Introduction

In pregnant women, clindamycin is used for various clinical conditions related to the mother or the neonate, especially when penicillin-allergy is encountered. Clindamycin is active against gram-positive cocci and anaerobic bacteria. In pregnant women at risk for endocarditis, it may be used to protect against infective endocarditis during labor[1]. Clindamycin is also one of the alternative drugs to protect neonates at risk for invasive group B streptococcal (GBS) disease[2]. Especially concerning the prevention of GBS disease, the use of antibiotics during labor has increased after the implementation of the culture-based prevention strategy in many countries[2].

During pregnancy and labor important physiological changes occur that may modify the pharmacokinetics of drugs. In non-pregnant individuals it has been shown that clindamycin distributes widely over the body, but that it does not adequately cross the blood-brain-barrier, even in case of bacterial meningitis[3]. It is metabolized and subsequently excreted into the urine and bile. The protein binding in non-pregnant humans ranges between 62% to 94%[4-8]. Because clindamycin is recommended in the prevention of both maternal and neonatal infection, the pharmacokinetics during labor in the mother and the transfer of the drug over the placental barrier are important.

Pharmacokinetic studies during labor face considerable ethical and logistical difficulties, limiting the opportunity for the collection of blood samples. These limitations may be overcome by the application of innovative approaches to the analysis of sparse data. Specifically, Non-Linear Mixed Effects Modeling (NONMEM) allows weighted analysis of data from both patients with large datasets and patients with small or incomplete datasets[9,10]. Moreover, by studying the population as a whole, the influence of specific circumstances on the individual PK parameters can be assessed using covariate analysis[11,12]. A more detailed background of population modeling can be found elsewhere[13,14]. The objective of this study is to describe the pharmacokinetics of intravenously administered clindamycin in pregnant women in the perinatal period and the transfer of clindamycin over the placental barrier.

Material and Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women with a gestational age of more than 26 weeks who needed antibiotic treatment with clindamycin were eligible for this study. Following the local guidelines, all women with proven or unknown *Streptococcus agalactiae* carriage were treated with

antibiotics when pregnancy was complicated by one of the following factors: preterm premature rupture of the membranes, rupture of the membranes for >18 hours, prematurity, fever (>37.8° C), bacteriuria in current pregnancy and a previous child with invasive GBS disease. The choice of the antibiotic for this study was dictated by the local guidelines, which recommend clindamycin in case of penicillin allergy in the prevention of GBS disease. Patients with an increased risk on endocarditis received clindamycin approximately 1 hour before delivery. Clindamycin is the antibiotic of first choice in the prevention of endocarditis, following local hospital guidelines.

The study was approved by the Medical Ethics Committee of the Medical Center Haaglanden, The Hague. Written informed consent was obtained from all patients. Women were excluded from the study when (1) they had been treated with oral or intramuscular antibiotics within 2 days before starting the therapy, (2) were unwilling to comply with the requirements of the study, (3) were known to be allergic to clindamycin, or (4) received co-medication that exhibits known interaction with clindamycin. All patients were at least 18 years of age.

All patients received a standard work-up that included a medical history and, biochemical and hematological examination at the onset of the study. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded before the antibiotic administration.

Drug administration and blood sampling

Before the administration of clindamycin two intravenous catheters were placed, one in each arm. Clindamycin was administered according to local guidelines in the hospital using the first catheter. The dose of 600 mg, as prescribed in the prevention of endocarditis, was administered over 20 minutes (12 ml/mL NaCl 0.9%) every 6 hours. The dose of 900 mg, as used in the prevention of GBS disease, was administered over 30 minutes (9 mg/mL NaCl 0.9%) every 8 hours. The exact duration of infusion was recorded.

Blood samples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 10 and 20 min (600 mg infusion) or 15 and 30 min (900 mg infusion). After completion of the infusion, sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30-45 minutes until the next antibiotic dosage. Blood samples were collected when possible, taking into consideration the physical and emotional inconvenience to the woman. The exact sampling times were recorded. Immediately after birth, both arterial and venous umbilical cord blood was obtained.

Blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 min. The supernatants were transferred into plastic storage tubes and frozen at -70˚ C until analysis.

Clindamycin HPLC

Samples were extracted by adding 0.05 ml NaOH (1.2 M) containing 40 mg/L propanolol as the internal standard. After vortexing, 2.6 ml dichloromethane (Sigma, The Netherlands) was added, and after vortexing, centrifuged during 5 min at 1500 g. The supernatant was removed and 2 ml pipetted in a new vial. The contents were dried at 40° C, airflow 5 L/min and solved in 0.2 mL potassiumdihydrophosphate, pH 4.6.

Clindamycin concentrations in 0.05 mL were determined by HPLC (Shimadzu, Den Bosch, NL). A reverse phase method (0.066 M pH 4.6 potassiumdihydrophosphate with 20% acetonitril), a C18 column (Bester, Amstelveen, NL) with a UV-VIS detector, wavelength 200 nm, temperature 40° C was used. The runtime was 10 min, injection volume 0.05 ml, flow 1 ml/min. A standard curve of clindamycin (Sigma-Aldrich, NL) was determined during each run. The lower limit of detection and quantification was 0.1 mg/L and was linear up to 50 mg/L. Higher concentrations were determined by diluting the samples. The between sample between day coefficient of variation (CV) was < 5%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method with INTERACTION. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM® software package (version VI, release 1.2, ICON Development Solutions, Ellicott City, Maryland, USA).

To determine the basic structural pharmacokinetic parameters various 2- and 3-compartment models were tested. Model selection and identification of variability were based on the evaluation of the mean objective function value (OFV), pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For differences between two structural models, the OFV with a pre-specified level of significance of $p<0.001$ was used (corresponding to a difference in OFV of at least 10 points). NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits, the goodness-of-fit plots were visually inspected. Data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero. Population values were estimated for the parameters clearance (CL), the volumes of distribution (V) and intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log-normal distribution. Therefore an exponential distribution model

was used to account for inter-individual variability. Possible correlation between inter-individual variability coefficients on parameters was estimated and if present accounted for in the stochastic model (NONMEM Omega block option).

Selection of an appropriate residual error model was based on the evaluation of OFV and inspection of the goodness-of-fit plots. A proportional error model, additive error model and a combined proportional-additive error model were tested to describe the residual variability between the observed concentrations and those predicted by the model. The residual error term contains all the error terms, which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters, on which a random effect has been identified, were plotted independently against the covariates bodyweight, body mass index, gestational age, oral temperature, the amount of edema and singleton or twin pregnancy to determine whether this influenced the pharmacokinetics. Covariate analysis was performed by forward addition of each candidate covariate into the model structure until no further improvement of goodness of fit was observed. A significance level of 0.05 was selected (corresponding to difference in OFV of 3.84 points). A further criterion for acceptance of covariate effects was that the estimated 95% confidence interval of the covariate effect did not overlap with zero. Contribution of each covariate to the final model was confirmed by backward deletion of each covariate from the model to account for possible interaction between covariates. Residual intra- and inter-individual variabilities were visually evaluated. The volume of distribution at steady state (V_{ss}) was calculated following standard procedures[15].

The accuracy of the final population model for the entire population was established using the bootstrap option in NONMEM, consisting of repeated random sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

The mean pharmacokinetic estimates of the final model derived from the PK analysis in NONMEM were used in Berkeley Madonna (version 8.3.5, Berkeley Madonna Inc, University of California, USA) to simulate the mean concentration-time profiles after a 600 mg and 900 mg clindamycin dose in pregnant women during labor. These maternal concentrations were used to calculate ratios of the umbilical cord concentrations and simultaneous maternal concentrations. Furthermore, ratios of the area under concentration curve for the free-drug in maternal serum for 24 hours over the MIC (fAUC$_{0\text{-}24h}$/MIC) were calculated, taking into account various percentages of clindamycin protein binding. For clindamycin the ratio for the total drug concentration should be at least 147, whereas for free-drug concentrations this ratio should be at least 27 [16].

Results

In total, seven patients were included. Of these patients four received clindamycin in the prevention of neonatal GBS disease and two to prevent the mother from endocarditis. One patient needed antibiotics to prevent both neonatal GBS disease and endocarditis. The physician decided to treat her with clindamycin using the dosing regimen for GBS prevention. Six patients with singleton pregnancies were in labor. One patient was treated because of preterm premature rupture of the membranes (PPROM) and had a twin pregnancy. The characteristics of the study population are presented in table 1. All patients receiving clindamycin as

Data	**Units**	**Mean**	**SD**	**Range**
Maternal age	Y	36.1	4.24	31.3-41.8
Gestational age	wk	38.3	3.01	34-42.3
Body mass index	kg/m^2	32.1	5.36	22.1-39.1
Weight	kg	86.1	14.2	59.5-104.8
Creatinin	umol/L	55.9	20.3	38-100
Ureum	mmol/L	3.39	1.17	1.8-5.6
Uric acid	mmol/L	0.31	0.086	0.18-0.43
AF	U/L	495	601	168-1794
ASAT	U/L	22.6	6.78	15-34
ALAT	U/L	10.9	5.27	5-18
γGT	U/L	10.7	6.85	5-25
LDH	U/L	351	74.6	247-445
Systolic blood pressure	mmHg	118	17.8	100-150
Diastolic blood pressure	mmHg	65	7.64	50-70
Pulse	/ min	84.6	11.1	74-108
Temperature	° C	36.9	0.23	36.7-37.4

Table 1 *Population characteristics (n=7).*

prevention of GBS were healthy. Of the three patients receiving clindamycin for endocarditis prophylaxis one had a minor stenosis of the mitral valve, the second had a prosthetic aortic valve, a prosthetic mitral valve and had been operated in the past on the tricuspidal valve. The third patient had a dysfunction of the aortic valve and autoimmune thrombocytopenic purpura (AITP), what had resulted in a splenectomy.

A total of 177 samples were included in the study. Two samples were excluded because the concentrations were <0.1 mg/L. In one patient it was not possible to place the two catheters each on a separate arm. Both catheters were placed on the right arm. The catheter used for the clindamycin infusion was flushed after the clindamycin administration and occluded. The samples collected during the clindamycin infusion in this patient were excluded from the analysis. In one patient with endocarditis prophylaxis, only four samples could be obtained due to obstruction of the sampling catheter. In three out of the seven patients results of an infusion in the postpartum period only for study purposes were also included. One patient receiving clindamycin as GBS prophylaxis received a postpartum dose of 600 mg, because she only agreed with a postpartum dose when the time interval between delivery and the last dose was short.

From all six patients in labor both arterial and venous umbilical cord blood samples were taken. The individual arterial and venous concentrations, the time-interval between the collection of the samples and the start of the antibiotic infusion

Patient	Clindamycin dose (mg)	Time between start infusion and sampling (h)	Time between maternal peak-concentration and sampling (h)	Arterial cord concentration (mg/L)	Venous cord concentration (mg/L)	Maternal concentration (mg/L)*
1	900	1.85	1.38	2.5	1.1	5.08
2	900	4.27	3.67	0.9	1.0	1.88
3	900	1.45	0.85	4.0	3.3	6.01
4	600	4.53	4.15	1.1	1.1	0.69
5	900	10.5	10	0.1	0.1	0.19
6	600	0.95	0.65	1.7	2.7	3.03

* Maternal concentrations were determined by simulation using Berkeley Madonna with the mean parameter estimates from the final PK model.

Table 2 *Clindamycin concentrations in arterial and venous umbilical cord blood and in maternal blood.*

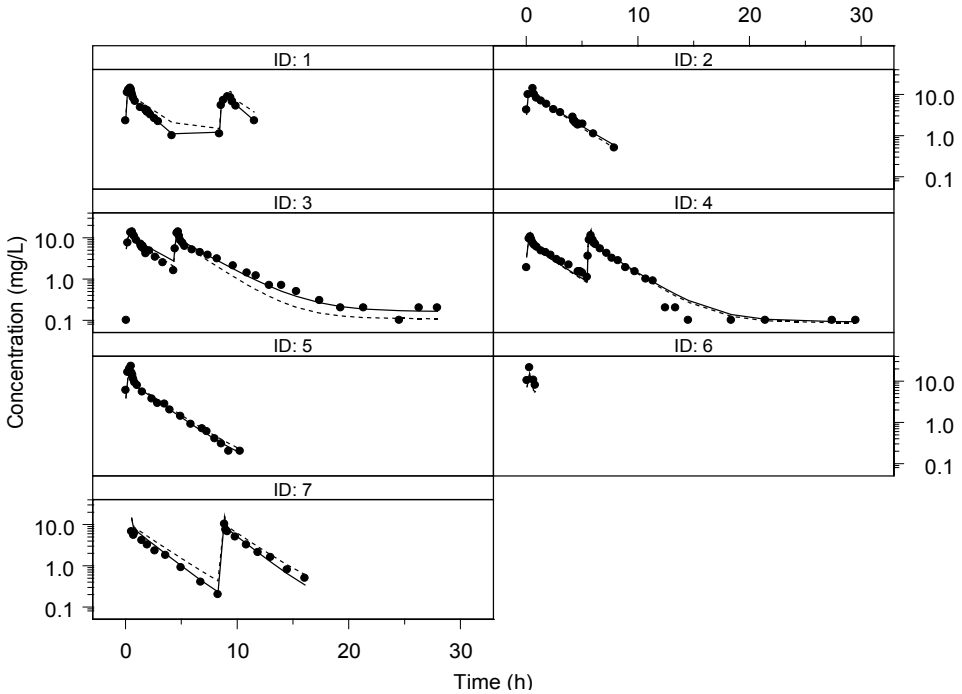

Figure 1: *individual plots with the final 3-compartment model. The black dots correspond with the individual datum points. The line represents the individual estimate and the dotted line the population estimate.*

and the time of the antibiotic peak-concentration are shown in table 2. Ratios of the venous umbilical cord blood concentrations and simultaneous maternal blood concentrations ranged from 0.22 to 0.89, with one outlier of 1.59. Ratios of arterial umbilical cord blood concentrations and simultaneous maternal blood concentrations ranged from 0.48 to 0.67 with one outlier of 1.59.

Various pharmacokinetic models were tested. Implementation of a 3-compartment model instead of a 2-compartment model improved the model-fit. The OFV decreased with 190 points, indicating that the 3-compartment model described the data better than the 2-compartment model. Using the 2-compartment model the concentration-time profiles in the two patients included for a prolonged period after the postpartum clindamycin infusion were not described adequately (figure 2A). Implementation of the 3-compartment model improved the model-fit, as is seen in figure 2B. Improvement of the model-fit using a 3-compartment model was also seen in the goodness-of-fit plots.

Considering the change in OFV, the visual inspection of the individual plots and the goodness-of-fit plots as well as the estimates of the pharmacokinetic parameters with their respective CVs, a three-compartment open model best

described the data. The residual error was best described by a proportional error model. Inter-individual variability was explained by variation in the parameters CL, V_3 and the residual error (54% on CL, 4.0% on V_3 and 55% CV on the residual error). None of the covariates could improve the model-fit. The V_{ss} was calculated to be 6.32 x 10^3 L and the gamma-phase $t_{1/2}$ was 2.6 h. The final estimates of the pharmacokinetic parameters and their respective CVs and 95% confidence intervals are presented in table 3. Due to the limited number of patients the parameter estimates for the inter-individual variability were not statistically significant. The individual plots and the plots of the observed concentrations versus the predicted concentrations are shown in figure 1 and 3 respectively.

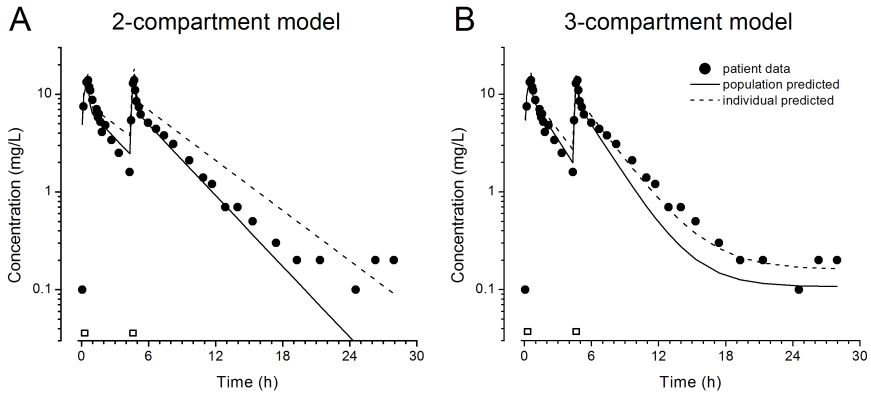

Figure 2*: ID 3 modeled with a 2- compartment model (figure 2A) and a 3-compartment model (figure 2B). The blocks indicate the time at which the infusion of the clindamycin was started and stopped.*

The bootstrap validation of the model of the entire population was performed with 100 runs. The bootstrap validation was successful for 92 runs. From the mean parameter estimates of the runs obtained from the bootstrap analysis only the estimate for V_3 deviated significantly from the predicted values from the NONMEM PK analysis. The estimates for V_3 could not be determined with good accuracy, because in the study population only two patients were included with a prolonged concentration-time profile. Therefore, this indicates that the accuracy of the final model is good.

Parameter	Units	Estimates of all patients		
		Mean	**CV**	**95% confidential interval**
Structural model parameters				
CL	L/h	10.0	41.2	1.92 – 18.1
V_1	L	12.4	11.5	9.62 – 15.2
V_2	L	52.2	6.3	45.8 – 58.6
V_3	L	6260	21.2	3650 – 8870
Q_1	L/h	137	6.27	120 – 154
Q_2	L/h	21.1	10.4	16.8 – 25.4
Variance model parameters				
interindividual variability in CL		0.293	69.3	-0.105 – 0.691
interindividual variability in V3		0.00162	920	-0.0276 – 0.0308
Interindividual variability in error		0.306	50.7	0.22 – 0.61
residual variability		0.0425	48.7	0.00193 – 0.0831

Table 3*: final estimates of the pharmacokinetic parameters and their respective CVs of the 3-compartment model.CL: Clearance, V_1: volume of distribution of the central compartment, V_2: volume of distribution of the first peripheral compartment, V_3: volume of distribution of the second peripheral compartment, Q: intercompartmental clearance between V_1 and V_2, Q_2: intercompartmental clearance between V_1 and V_3,CV: coefficient of variation.*

In the prevention of neonatal GBS disease clindamycin is administered intravenously to the mother. The clinical breakpoint of clindamycin for GBS as determined by the EUCAST is 0.5 mg/L[17]. Using the dosing regimen of 900 mg every 8 hours and 600 mg every 6 hours, the values of $fAUC_{0\text{-}24h}$/MIC for protein binding ranging from 60% to 95% are shown in table 4. The ratio of $fAUC_{0\text{-}24h}$/

MIC for the total drug concentration is also shown in table 4. Taking into account the protein binding, the 900mg dosing regimen results in a ratio of at least 32 for a protein binding up to 80%. The limited difference in ratio of $fAUC_{0\text{-}24h}$/MIC for the total drug concentration in maternal serum indicates that the dosing regimen of 900 mg every 8 hours might be more adequate than the dosing regimen of 600 mg every 6 hours.

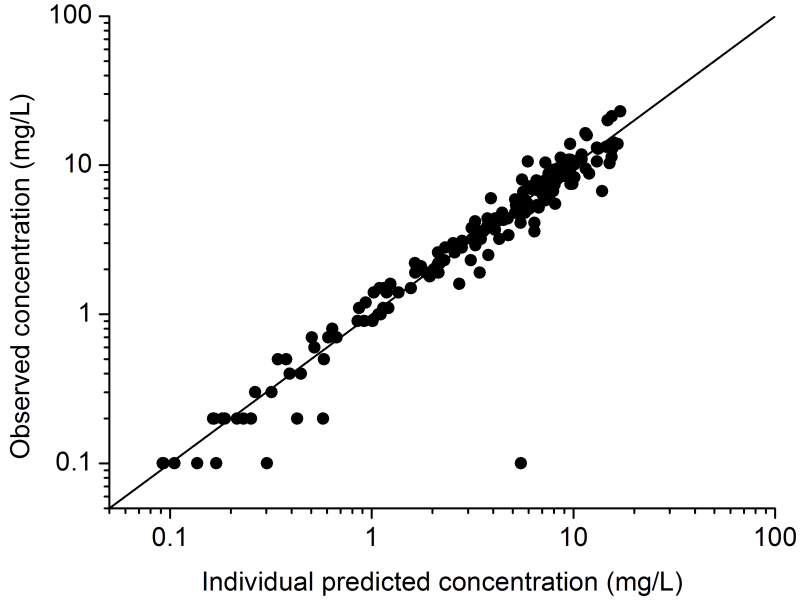

Figure 3: *plot of the observed versus the predicted concentrations using the final 3-compartment model.*

Discussion

In this study a pharmacokinetic model was developed to describe the pharmacokinetics of clindamycin in pregnant women. The pharmacokinetics of clindamycin in pregnant patients is best described using a 3-compartment model. Clearance and gamma-phase half-life were 10.0 L/h and 2.6 h, respectively. For the average pregnant women, the $fAUC_{0\text{-}24h}$/MIC ratio ranges from 64 to 8 for assumed

Percentage of protein binding	600 mg every 6 hours	900 mg every 8 hours	Minimal value for efficacy[16]
Total concentration (free and bound clindamycin)	129	159	147
60%	51.7	63.6	27
70%	38.7	47.8	27
80%	25.8	31.8	27
85%	19.4	23.9	27
90%	12.9	15.9	27
95%	6.46	7.98	27

Table 4: *ratios of $fAUC_{0\text{-}24h}$/0.5 for different percentages of protein binding and two dosing regimens. The MIC value for GBS used (0.5 mg/L) is determined by the EUCAST[17]. Additionally, the minimal value for efficacy as reported in the literature is shown.*

protein binding percentages of 60 to 95% using the 900 mg dosing schedule.

Data on the pharmacokinetics of clindamycin in pregnant women and non-pregnant individuals are scarce. Two previous studies determined the ratio of venous umbilical cord blood and maternal blood[18,19]. Weinstein et al found a ratio of 0.46[18] after intravenous administration during caesarean section. In contrast, Philipson et al[19] found ratios of 0.18 and 0.25 after an oral dose of clindamycin in women with a gestational age of 10 – 22 weeks during a therapeutic abortion. Our values are comparable to the ratio reported by Weinstein et al[18]. The low values reported by Philipson et al might be explained by the low gestational age and the difference in route of administration (orally in the study by Philipson and intravenously in our study). The pharmacokinetic parameter estimate in the mother for clearance using our 3-compartment model, results in a lower value as compared to values reported in the literature (10.3 L/h for our study versus 19.8 – 26.4 L/h in the literature[18,20-23]).

In the final 3-compartment model all structural parameters were estimated with an adequate precision (i.e. CV <51%). When the data were analyzed with a 2-compartment model, the estimates for the volumes of distribution could not be determined with adequate precision. As could be explained by the presence of the third compartment, the estimated value for the clearance using a 2-compartment model was larger than using the 3-compartment model (respectively 28.3 L/h and 10.0 L/h). This supports the inclusion of a third compartment in the pharmacokinetic model.

Clindamycin in mainly bound to alpha1-acid glycoprotein, an acute phase protein. The protein binding is dependent on the serum concentration of both alpha1-acid glycoprotein and clindamycin[8]. High concentrations of alpha1-acid glycoprotein result in a high protein binding, whereas, due to non-linearity in the protein binding an increase in the clindamycin concentration leads to a decrease in protein binding[8]. In our patients the percentage of protein binding is unknown, but compared to non-pregnant healthy volunteers it is likely to be reduced due to the state of pregnancy, but possibly increased due to being in labor, stress or the presence of infection[24]. Since only the free unbound fraction of drugs is active and the plasma protein binding of clindamycin is relatively high, a minor increase in protein binding might influence its efficacy.

Clindamycin has a time-dependent action in vitro, but clinical efficacy is more closely related to $fAUC_{0\text{-}24h}$/MIC[16,25]. The therapeutic goal to achieve a static effect is a ratio of at least 27, taking into account the clindamycin protein binding. However efficacy might be increased with higher ratios. Not for all percentages of protein binding reported in the literature, the current dosing regimen reaches adequate ratios. Furthermore, these concentration time profiles are only applicable for the average pregnant women. When one would take into account the inter-individual variability in pharmacokinetics this regimen is likely to be inadequate for some pregnant women.

Furthermore, to prevent neonatal GBS disease, both concentrations in maternal and in fetal serum have to be adequate. The concentration of alpha1-acid glycoprotein in the neonate increases with gestational age[24]. As has been shown for alprenolol, the affinity of $alpha_1$-acid glycoprotein for clindamycin might be decreased in the first 7 days of life, partly due to displacement by bilirubin[24]. Peak concentrations in the fetus are likely to be lower compared to the maternal peak concentrations, as has been shown for amoxicillin previously[26]. Arterial umbilical blood directly originates from the fetus and therefore these concentrations represent concentrations in the fetus. The number of measured clindamycin concentrations in the arterial umbilical cord samples in our study is relatively low. When the protein binding and the inter-individual variability are taken into account, it is doubtful whether adequate concentration-time profiles are reached in the fetus.

In conclusion, these data indicate that for the average pregnant women the current dosing regimen reach adequate concentrations assuming that the protein binding will not exceed 80% of the total concentration. To prevent the fetus from infection, concentrations in fetal blood have also to be adequate. Unfortunately, these data suggest that the concentration-time profiles in the fetus might be inadequate, at least for a substantial part of the population. More pharmacokinetic studies including data both of the mother and of the neonate are needed to investigate whether the currently advised dosing regimen is adequate to use as preventive measure against neonatal GBS disease.

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Chapter 11

Pharmacokinetics of penicillin G in infants with a gestational age of less than 32 weeks.

Anouk E. Muller, Joost DeJongh, Ymka Buļt, Wil H.F. Goessens, Johan W. Mouton, Meindert Danhof, John N. van den Anker

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Abstract

The pharmacokinetics of penicillin G was studied in 20 preterm neonates with a gestational age of less than 32 weeks on day 3 of life using a population approach performed with the nonlinear mixed effects modeling program NONMEM. The derived population estimates and the correlation matrix of these estimates were used to perform Monte Carlo Simulations (MCSs) and obtain the probability of target attainment (PTA). Penicillin G pharmacokinetics was best described by a two-compartment pharmacokinetic model. The population estimates of the central volume of distribution, peripheral volume of distribution, intercompartmental clearance and the total body clearance were 0.359+/-0.06 L, 0.152+/-0.03 L, 0.774+/-0.28 L/h and 0.103+/-0.01 L/h (mean +/-SE), respectively. The terminal $t_{1/2}$ was 3.9 h. Clearance increased significantly with increasing birth weight. Assuming the percentage of time that the concentration of unbound drug remained above the MIC (%*f*T>MIC) of 50% for preterm neonates, the susceptibility breakpoint based on a 100% PTA was ≤4 mg/L simulating the current dosing regimen of 50.000 U/kg every 12 h. This regimen is therefore adequate for the treatment of common neonatal infections on the third day of life.

Introduction

Penicillins are important antimicrobial agents in treatment of bacterial infections in newborn infants, with a high clinical efficacy in eradicating common pathogens in combination with a high degree of safety. Because of lack of evidence from randomized clinical trials in favor of any particular antibiotic or antibiotic regimen[1], penicillin G remains important among the antibiotics most commonly given to neonates in the treatment of presumed early neonatal sepsis.

Infectious complications during the immediate postnatal period are not uncommon and require prompt antibiotic treatment[2-5]. Differences in body composition and organ function can significantly affect the pharmacokinetics in neonates. In very premature neonates (i.e. gestational age of less than 32 weeks) the disposition of antibiotics may differ from full term neonates as a result of differences in absorption, distribution, biotransformation and excretion[6-8]. As a result, dose estimation on the basis of body size or allometric scaling may be inadequate. Specifically, in very premature neonates maturation processes of the organs may influence the relevant pharmacokinetic parameters.

The efficacy of the penicillins is primarily correlated to the percentages of time that concentrations of unbound drug remained above the MIC (%*f*T>MIC)[9-11]. In general, the therapeutic goal to cure infections caused by Gram-positives is a %*f*T>MIC of at least 40% of the antimicrobial, which corresponds to an in vivo static effect in animal studies[12]. Studies showing a clear relationship between exposure and efficacy in premature neonates are not available. Because prematures have to be regarded as immunocompromised, to our opinion it is reasonable that in this patient group the exposure should correspond to exposures that correlate to a 1 to 2 log drop of CFU in various models, and thus be bactericidal rather than bacteriostatic. Thus, for penicillins, this percentage should be at least 50%[13]. Since the goal of treatment is to attain this target for every individual in the population, the dosing regimen in this age group should be defined taking the inter-individual pharmacokinetics variability into account. Monte Carlo Simulations (MCS) is a technique that is commonly used to determine the probability of achieving therapeutic concentrations on the basis of population pharmacokinetic parameter estimates and their measures of dispersion[14-19]. We investigated the pharmacokinetics of penicillin G and the adequacy of the dosing regimen in very premature neonates on the third day of life to allow us to construct a population pharmacokinetic model and to use the parameter estimates to perform MCS in this specific age group. We then used that information to define optimal dosing regimens.

Materials and methods

Patients and treatment

Preterm neonates with suspected or documented septicemia or invasive infection were eligible for this study. The neonates were hemodynamically stable (diuresis > 1 mL/kg/h; systolic and diastolic blood pressure above the third percentile adjusted for gestational age), had a normal liver function, had not received inotropic or nephrotoxic drugs, did not have an intracranial hemorrhage beyond grade II, and had an indwelling arterial catheter for clinical purposes. The partial pressure of oxygen in arterial blood was kept at greater than 50 mmHg or oxygen saturation between 87% and 92%, and hematocrit values were maintained above 0.32 by packed erythrocyte transfusions. Neonates were excluded from the study if they had life-threatening illnesses or became hemodynamically unstable (systolic and diastolic blood pressure below the third percentile adjusted for gestational age; diuresis < 1 mL/kg/h). Also excluded were infants with severe asphyxia, defined as having: profound umbilical artery acidemia (pH < 7.00), persistence of an Apgar score of 0 to 3 longer than 5 minutes, neonatal neurological sequelae (e.g., seizures, coma, hypotonia), and multiorgan system dysfunction (e.g., cardiovascular, gastrointestinal, hematologic, pulmonary or renal). Subjects were enrolled after parental informed consent. During that period, empirical treatment consisted of penicillin G or amoxicillin in combination with tobramycin or cefotaxime. Patients treated with penicillin G were eligible for this study.

Penicillin G was administered as an intravenous bolus injection of 50.000 U/kg every 12 hours. From each subject leucocytes-count, platelets-count, blood and superficial cultures were performed as part of their routine work-up.

Pharmacokinetics study

The pharmacokinetics of penicillin G was studied on day three of life. Blood samples (200 µL) were taken from an indwelling arterial line just before the administration of an intravenous bolus dose and at 0.03, 0.5, 1, 2.5, 4, 8, 12 h after administration. A 24 h sample was taken from those patients that did not receive a subsequent dose. Samples were immediately centrifuged in a microcentrifuge (Merck-type Eppendorf 5414: 3.000 x g) for 1 minute and serum was stored at -70° C.

Penicillin G HPLC assay

Chromatographic analysis was performed with a glass-prepacked column (100 by 3 mm) containing ODS-2 Chromospher Spherisorb beads (5-µm-diameter particle size; Chrompack, Middelburg, The Netherlands) combined with a guard column. A Bio LC pump (model 410, Perkin-Elmer, Norwalk, Conn.) was used to deliver the eluent consisting of 16% (vol/vol) acetonitrile and 50 mM sodium phosphate buffer (pH 6.9) at a flow rate of 0.8 ml/min. The separations were carried out at room

temperature. The eluate was monitored with a Perkin-Elmer LC-95 UV/visible spectrophotometer detector at a wavelength of 215 nm. As an internal standard 25 µg/ml methicillin in 100 % methanol (vol/vol) was used. Briefly, hundred µl of the internal standard was added to a 100 µl aliquot of the serum sample. This mixture was immediately vortexed for 30 seconds. Subsequently, the sample was kept for 10 min at -20 °C, again vortexed for 30 seconds and finally centrifuged at 1,500 g for 10 min at room temperature. The supernatant was filtered (millipore) and 10 µl was injected onto the column.

HPLC-grade acetonitrile was purchased from Rathburn (Walkerurb, Scotland). The other chemicals were purchased from Aldrich-Chemie (Steinheim, Germany). All chemicals applied were of the highest grade commercially available.

The lower limit of detection of penicillin was 0.5 µg/ml. The coefficients of inter-assay variation determined at concentrations of 100 and 20 µg/ml were 2.6% and 2.3%, respectively. The intra-assay values were 0.75% and 1.05%, respectively.

Pharmacokinetics analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect (population) Modeling (NONMEM). This approach estimates the structural PK parameters considering both inter-individual variability within the population and the intra-individual (i.e. residual) variability. The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the first-order conditional estimation (FOCE) method with interaction option. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM version V (NONMEM project group, University of California, San Francisco, USA).

To determine the basic structural pharmacokinetic parameters several models were evaluated. One, two, and three-compartment models were tested and evaluated for goodness-of-fit. Model selection and identification of variability was based on the likelihood ratio test, pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For the likelihood ratio test on differences between two models, the objective function value (OFV) with a pre-specified level of significance of $P<0.001$ was used. NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits the goodness-of-fit plots were visually inspected. The data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero.

The stochastic part of the model was selected to describe inter-individual

variability in the pharmacokinetic parameters and assumed a log normal distribution of all model parameters over the population. Therefore an exponential distribution model was used to account for inter-individual variability:

$P_i=\theta * \exp(\eta_i)$,

in which P_i is the individual value of the model parameter P, θ is the population estimate for parameter P and η_i is the normally distributed inter-individual random variable with mean zero and variance ω_2. Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots. The model was modified to objectively account for unexplained inconsistency in the data. To reduce the influence of neonates with large unexplained inconsistency in the concentration-time profile on the population estimates, these neonates were objectively determined by means of the residual error and weighted less in the estimates of the population.

To refine the stochastic model covariate analysis was also performed. The estimated pharmacokinetic parameters were plotted independently against the covariates gestational age, birth weight, gender and the presence of a dosing history to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance using the likelihood ratio test and the residual intra- and inter-individual variability were visually evaluated. A covariate was retained in the model if it produced a decrease in objective function of > 10.8 ($p<0.001$). In addition, we investigated whether there were significant differences in the pharmacokinetics between neonates with a birth weight of less than 1000 gram and neonates with birth weight of more than 1000 gram. V_{ss} and $t_{1/2}$ were calculated following standard procedures[20].

Estimation of *f*T>MIC and Monte Carlo Simulations

The estimates of the pharmacokinetic parameters and measures of dispersion were used to simulate various dosing regimens and obtain %*f*T>MIC as a function of MIC[19]. Protein binding was estimated at 40% ± 2.5%[21]. The protein binding of penicillin G in premature neonates is unknown. However, protein binding in neonates is generally lower compared to adults. It is therefore likely that the estimated protein binding of 40% overestimates the not-active protein bound fraction of penicillin G in these neonates. The use of 40% is therefore a conservative estimate. MCS was performed using the MICLAB version 2.36 program (Medimatics, Maastricht, the Netherlands) simulating 10.000 subjects for each regimen. The program allows inclusion of the covariance matrix (or correlation matrix) of the parameter estimates used in the simulations. The output consisted of a probability distribution, a cumulative probability distribution, and specific confidence intervals over user defined MIC and %*f*T>MIC ranges.

Results

Demographic data.

Twenty neonates with a gestational age under 32 weeks were included in the study. Demographic, laboratory and clinical parameters are shown in table 1. The average weight was 1195 g (range 650-2030 g). Half of the subjects were born from mothers with preeclampsia or HELLP-syndrome. Other reasons for premature birth were: suspected intra-amniotic infection, preterm contractions with meconium stained amniotic fluid and PPROM.

Parameter	**Mean**	**SD**	**Range**
Gestational age (wk)	29 5/7	1 5/7	26 3/7- 32 0/7
Gender (M/F)	12/8		
Weight (g)	1195	387	650-2030
Hematocrit (%)	46	7	33-63
Leucocytes (10^3/mm^3)	15	13	5-54
Platelets (10^3/mm^3)	203	103	74-497
Creatinine	46	17	10-82
APGAR 1 minute	6	3	1-10
APGAR 5 minutes	8	1	6-10
Artificial ventilation (Yes/No)	9/11		
No. of positive bloodculture	0		
No. of positive superficial culture	1 (*Streptococcus agalactiae*)		

Table 1: *Demographic, laboratory and clinical parameters of 20 patients studied on day three after birth.*

Population pharmacokinetics

167 samples were included in the pharmacokinetic analysis. In 11 neonates samples were obtained only in the first 12 hours after the i.v. bolus and in 9 neonates a sample could be obtained after 24 hours. A bi-phasic rate of decline in the penicillin G concentration versus time (i.e. a two-compartment model) best described the data using a combined error model with an additive and proportional error. Figure one shows the observed concentrations in the individual patients as well as the predicted concentration time curves as obtained from the final model, while figure 2 shows the observed concentrations versus the individual predicted concentrations for the whole population. The distribution around the reference line of perfect prediction was symmetric with a correlation coefficient of 0.80, but deviates significantly from one. The pharmacokinetic parameter estimates are shown in table 2. The percent coefficient of variation reflects both the inter-individual or intra-individual

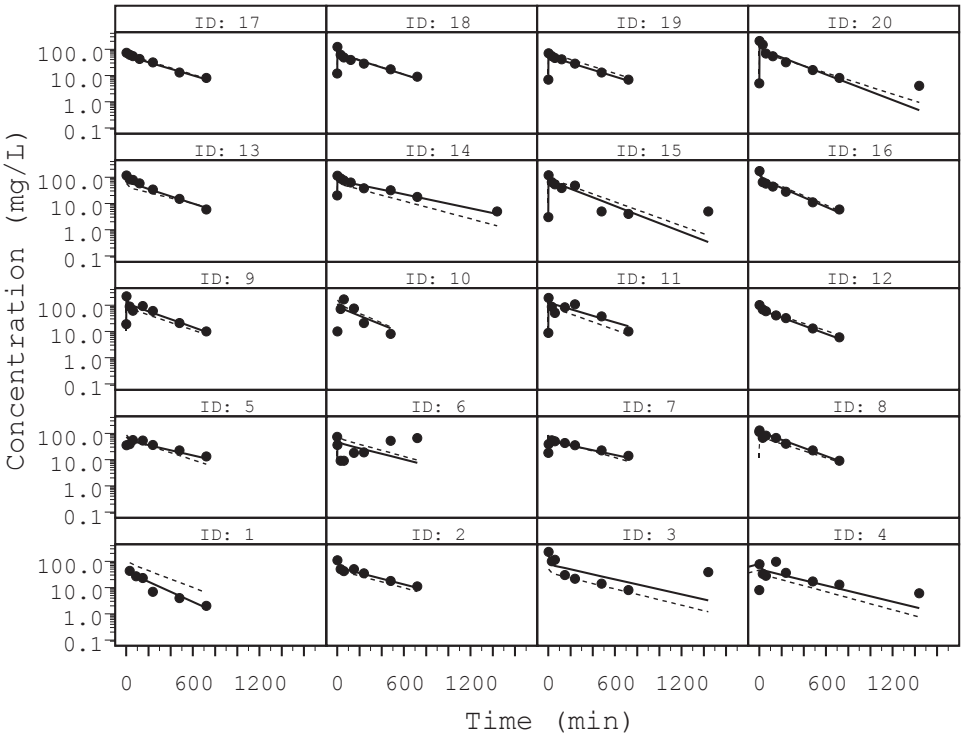

Figure 1: *Individual plots of the 20 very preterm neonates. The black dots correspond with the individual datum points. The line represents the individual estimate and the dotted line the population estimate. Neonate 1, 3 and 6 were weighted less in the population estimation.*

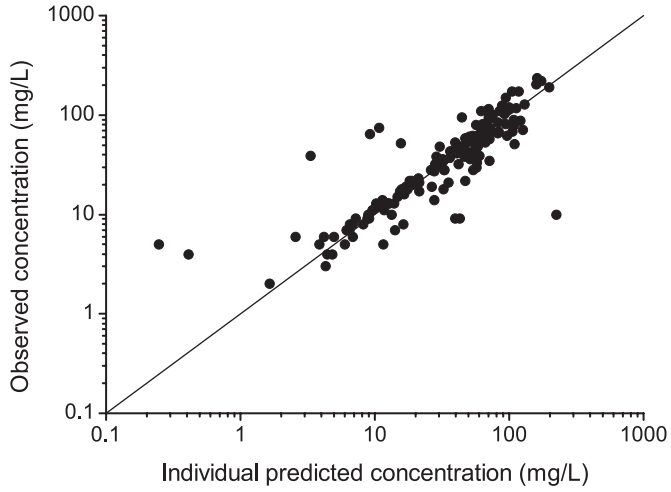

Figure 2: *Plot of individual predicted versus observed concentrations of penicillin G for 20 patients. The correlation coëfficient was 0.801. The individual datum points for the entire population and the x=y line is also shown.*

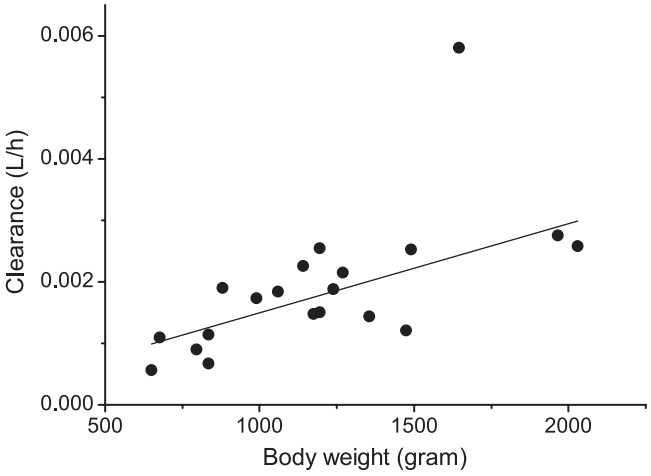

Figure 3: *Observed relation between body weight and clearance.*

Parameter	Mean value	Standard error
Structural model parameters		
CL (L/h)	0.103	0.0104
V_1 (L)	0.359	0.0558
V_2 (L)	0.152	0.0312
Q (L/h)	0.774	0.277
Variance model parameters		
Interindividual variability in CL	0.164	0.0865
Interindividual variability in V_1	0.39	0.126
Residual variability (proportional component)	0.104	0.0316
Residual variability (additive component)	1.12	0.891
Derived pharmacokinetics parameters		
V_{ss} (L)	0.540	-
$t_{1/2\beta}$ (h)	3.9	-

Table 2: *Pharmacokinetic parameters of penicillin G in 20 preterm neonates.*

variability of the pharmacokinetic parameters. Estimation of inter-individual variability was possible for the parameters CL corrected for body weight, V_2 and the infusion rate (34.5 % for CL corrected for body weight, 17.1% for V_2 and 89.9% for the infusion rate). The residuals were generally small, but the additive error of neonate 1, 3 and 6 differs 2.5 to 3.5 times from the median of these neonates.

We examined the relationship between CL and gestational age, gender, body weight and the presence of a dosing history (i.e. whether the neonate had received a previous dose of penicillin or not). There were no significant correlations between CL and gender or the presence of a dosing history. CL increased significantly with an increasing body weight (Figure 3, $p<0.01$). Incorporation of body weight on

clearance in the model improved the model fit significantly. There was a significant correlation between gestational age and body weight ($p<0.01$). Incorporation of the gestational age did not further improve the model fit. No significant differences in pharmacokinetic parameter estimates could be demonstrated between neonates with a birth weight of more and less than 1000 gram.

To determine the probability of target attainment (PTA) for the dosing regimen used, MCS was performed. Assuming a %*f*T>MIC of 50% for preterm neonates, a PTA of 100% was reached with the currently recommended dosing regimen of 50.000 U/kg every 12h for pathogens with MICs of ≤4 mg/L. Figure 4 shows the %*f*T>MIC for the dose of 50.000 U/kg and 3 different dosing intervals based on mean population parameter estimates and the correlation matrix of these estimates.

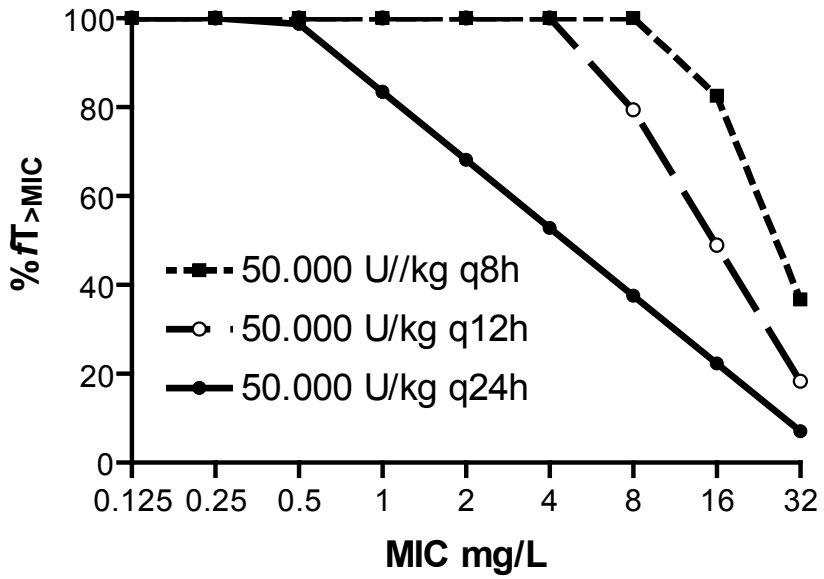

Figure 4: *Percent of time the unbound fraction of penicillin G remained above the MIC (%fT>MIC), based on the pharmacokinetic estimates and the correlation matrix of the parameter estimates, as a function of the MIC for 3 regimens.*

Discussion

Pharmacokinetics of penicillin G in neonates with a gestational age of less than 32 weeks was best described by a two-compartment model, yielding estimates of the terminal $t_{1/2}$ of 3.9 h, V_{ss} of 0.54 L and CL of 0.103 L/h. The dosing regimen of 50,000 U/kg every 12 h is adequate for the treatment of neonatal infections caused by common microorganisms on day 3 of life.

Most studies on the pharmacokinetics of penicillin G in neonates have been performed after intramuscular administration[22-24]. However, the intramuscular route should be avoided, because this may result in erratic absorption in the sick, infected newborn with restricted blood supply to the extremities[25]. Mulhall et al.[24] performed a study on the pharmacokinetics of penicillin G after intravenous administration in 4 neonates with a gestational age ranging from 27 to 40 weeks and found a CL of 0.12 +/- 0.07 (mean +/- SD) L/h/kg, V of 0.61 +/- 0.28 L/kg (mean +/- SD) and $t_{1/2}$ of 3.8 h. These results are similar to our data.

Other penicillins have larger V and longer terminal $t_{1/2}$ in prematures, especially when compared with adults[13]. V varied from 0.3 L/kg for ampicillin to 0.41-0.68 L/kg for amoxicillin in neonates[26-28], compared to 0.45 L/kg found for penicillin G in our study. The terminal $t_{1/2}$ of penicillin G in neonates with a gestational age of less than 32 weeks was longer compared with the value of 0.5h as reported for healthy adults[21], but is in the same range as the values of between 2 to 9.5 h that have been reported for other penicillins in neonates[13,26-30].

Within the limited number of samples available from patients in this age group, there is some unexplained inconsistency in the data. This might be caused by subcutaneous administration of penicillin G, erratic sampling times, or accidental exchange of samples. Three neonates had large unexplained inconsistency in the data based objectively on their residual error and were therefore weighted less in the population estimates. Using this method none of the neonates were excluded from the study and all data were used in the analysis. Consequently, there is a deviation between the line of identity and the regression line of the observed versus predicted concentrations, indicating that the description of the pharmacokinetics in this age group can be improved. To this end more data are needed.

The presence of a third elimination phase ($t_{1/2,\gamma}$) for penicillin G has been described previously, both in animal models as well as in adult humans[21,31]. Our data could not be described by a three-compartment model. But the terminal elimination phase ($t_{1/2,\beta}$) found in our study, was comparable to the $t_{1/2,\gamma}$ of 3.1 h in human adults[21]. Both the limited number of samples taken in the initial distribution phase and the unique body composition of the neonate, comprising approximately 75% water, complicate the distinction between the initial distribution and second elimination phase. Possibly, the initial phase in our study represents both the initial and second phase as found in the study of Ebert et al[21]. Thus, the slow elimination

we found may represent the third elimination phase for penicillin G as determined in that study.

Inter-individual variability was partly explained by variation in CL, V_2 and the infusion rate. The variability in infusion rate represents not only the variation in rate of the manually administered intravenous bolus injection, but also variation in the sampling times between the first samples. Especially for the first samples the exact sampling times are crucial.

Growth and development are major aspects in infants, therefore both size and gestational age may have an impact on the prediction of CL[32]. Maturation of CL begins before birth, suggesting that gestational age would be a physiologically appropriate covariate to explain the time course of changes in CL[32]. In our data changes in CL were best explained by differences in body weight. Probably because both gestational age and other factors influencing the development of renal function are represented by an adequate increase of body weight in time. Furthermore, the range in gestational age as included in our study was relatively small. While CL slightly increased as a function of birth weight for the entire group, differences between subgroups with a birth weight of more and less than 1000 gram could not be demonstrated. The analysis of correlations between covariates and pharmacokinetic parameters estimates is more sensitive when all neonates are included in the study.

The enhanced inter-individual pharmacokinetics variability in prematures complicates the calculation of the therapeutic dosing regimen. The dosing regimen should be adequate for the entire population. We concluded that the 100% PTA obtained with simulation of the recommended regimen was adequate to treat neonatal infection on the third day of life. In case of meningeal involvement effective concentrations are required in the cerebrospinal fluid (CSF). Little is known on the pharmacokinetics of penicillin G in the CSF of premature neonates. We do not know what percentage of penicillin G penetrates the CSF in premature neonates with meningitis. Given the low MIC of GBS to penicillin (up to 0.12 mg/L), with use of the currently recommended dosing regimen it is sufficient when the penetration of penicillin into the CSF is at least 3%.

Shortening the dosing interval to 8 h, will not have additional value in treatment of infections in neonates with a gestational age of less than 32 weeks. When these infections are caused by microorganisms with low MICs, like *Streptococcus agalactiae*, a regimen with a prolonged dosing interval of 24 h, is also likely to have clinical success. However, for empirical therapy this regimen is suboptimal.

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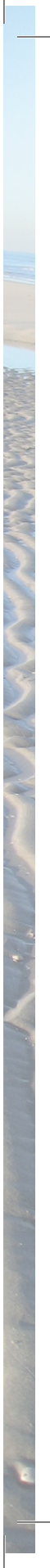

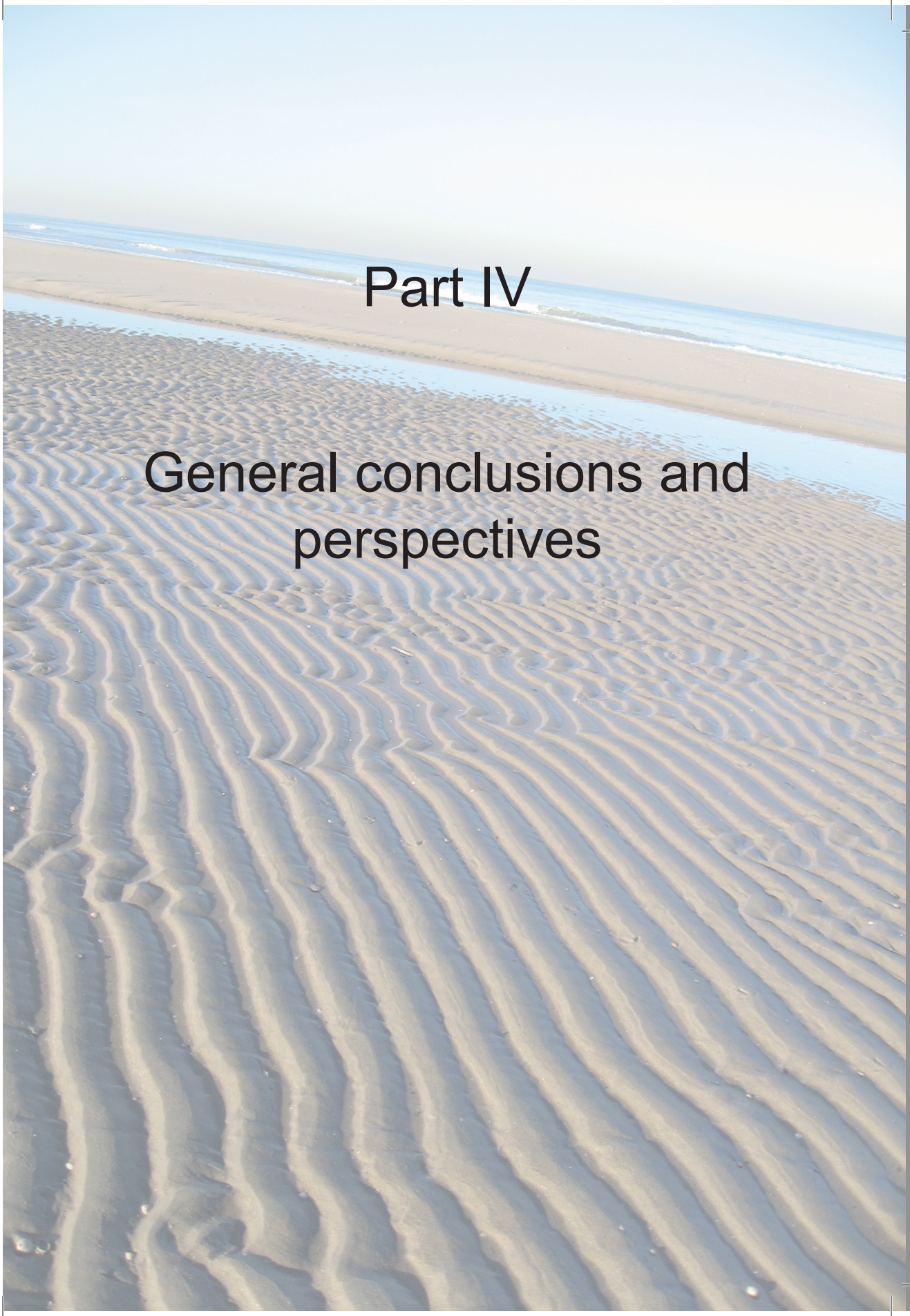

Part IV

General conclusions and perspectives

Chapter 12

Population pharmacokinetics of antibiotics to prevent group B streptococcal disease:

Summary, conclusions and perspectives.

Introduction

In the peripartum period infections due to group B streptococci (GBS) play an important role in infectious morbidity and mortality. In the 1970s GBS emerged as leading cause of neonatal morbidity and mortality[1]. Neonates suffering from GBS disease usually present with pneumonia, sepsis and / or meningitis. Less appreciated is the fact that GBS can also cause a variety of maternal clinical infections in the peripartum period. Apart from cervicovaginal colonisation, which is usually asymptomatic, GBS can cause urinary tract infections, vulvovaginitis, intra-amniotic infection, mastitis, bacteremia, sepsis, meningitis, endometritis and wound infections. These infections often have an impact on both the mother and her child (as reviewed in **Chapter 3**).

During the last decades strategies to prevent neonatal GBS disease were implemented in several countries[2]. The cornerstone of these preventive measures is the administration of antibiotics shortly before and/or during labor. To recommend the use of these antibiotics one has to weigh the benefits and the unintended consequences. Overall, most incidence figures of culture-proven GBS disease in the first 7 days of life have decreased, suggesting that antibiotics to be effective[2]. However, these incidence figures are influenced by multiple factors[3]. Furthermore, neonatal GBS disease has also been reported in neonates whose mothers did receive antibiotic prophylaxis. Therefore, concerns are raised on the antibiotic prophylaxis itself. A specific question is whether current guidelines recommend the optimal antibiotic dose and dosing interval. Moreover, although unintended consequences for the mother, such as an anaphylactic shock, are rare, nowadays there is growing concern for potential, unintended consequences for the neonate. Antibiotic use during labor might influence the initial bacterial flora in neonates. A changed initial flora might have impact on the development of the immune system of the neonate[4,5]. Meanwhile there is moderate evidence on the efficacy of the antibiotics and little attention for the unintended consequences of antibiotic use during labor, guidelines recommend this prophylaxis to up to 35% of all pregnant women[2] (as reviewed in **Chapter 2**).

To study the efficacy of antibiotics and to search for the optimal dosing regimen used in the prevention of GBS disease, knowledge on the pharmacokinetics is needed. Pharmacokinetics is the discipline that applies mathematical models to explain and predict the time-course of drug concentrations in the body. Important determinants of the time-course of the drug concentrations are the extent and rate of absorption, distribution, metabolism and excretion.

Pharmacokinetic studies in pregnancy: general approach

Antibiotics in the prophylaxis of GBS disease are administered to the mother, while the antibiotics are also meant to protect the fetus. Since fetal antibiotic concentrations are reached from the maternal circulation, adequate concentration-time profiles in the mother are essential and therefore a first issue in pharmacokinetic studies during pregnancy. In pregnant women there are changes in the absorption, distribution, metabolisation and excretion of drugs. For example, in pregnancy the plasma volume increases, while albumin protein binding decreases and the blood flow to the liver as well as the glomerular filtration rate rise as well[6]. Because all these adaptations occur simultaneously and change with advancing gestational age, extrapolation of pharmacokinetic data from non-pregnant individuals is difficult.

When the administered antibiotics reach an adequate concentration-time profile in the pregnant woman, the second issue is the transfer of the antibiotic over the placental barrier. In this way the antibiotic is able to reach the fetus. The rate of transfer over the placental barrier is related to the maternal-fetal concentration gradient and is inversely proportional to the permeability of the placental membrane[7]. The thickness of the placenta increases with gestational age and in various disease states, like diabetes and hypertensive disorders complicating pregnancy[8]. Antibiotic concentrations determined in venous umbilical cord serum samples can provide information on the transfer over the placental barrier. Unfortunately, postpartum blood samples from the umbilical cord can be obtained only once per patient and also at a time point beyond the control of the investigator.

As a third step, the pharmacokinetics in the child need to be studied. This can partly be achieved using blood samples of the arterial umbilical cord after birth and those of the neonate. It is important to realize that there will be differences in pharmacokinetics in the child before and after birth. In the fetus, blood flow towards the mother via the arterial umbilical cord to the placenta will be the main route of excretion of drug from the neonate. After birth, the only routes of excretion of drugs are through the excreting organs of the neonate. Furthermore, the blood flow will redistribute after birth, what might influence the pharmacokinetics.

In conclusion, to study the efficacy of the GBS prophylaxis pharmacokinetics in both mother and neonate has to be determined. In contrast to maternal blood samples, which can be obtained relatively frequently umbilical cord samples can be obtained once for each patient. This will result in unbalanced study groups. Fortunately, pharmacokinetic data can nowadays be analyzed with a so-called "population approach" in computer programs such as NONMEM (Non-linear Mixed effects Modeling). Using this approach, data of the whole population are simultaneously analyzed while taking into account inter-individual and intra-individual variability. Data of unbalanced groups and sparse data can be analyzed in a meaningful manner by using a population analysis[9,10].

While the availability of pharmacokinetic data is an important prequisite to evaluate the prophylaxis, the relationship between pharmacokinetic properties and clinical effect is also very important. During pregnancy and delivery several antibiotics are used. One of the antibiotics frequently used in Europe in the prevention of neonatal GBS disease is amoxicillin. In some hospitals amoxicillin combined with clavulanic acid is used in the prevention of neonatal infection, including the prevention of GBS disease, because this combination is also active against amoxicillin-resistant *Escherichia coli*. When penicillin allergy is encountered, clindamycin is often used as an alternative. The relationship between pharmacokinetic properties, microorganism susceptibility (as indicated by the minimum inhibitory concentration (MIC)) and clinical effects has been increasingly well understood. The efficacy of the penicillins, like amoxicillin, is primarily correlated to the percentage of time that the serum concentration of the unbound drug remains above the MIC (%fT>MIC)[11-13]. Similar to amoxicillin, clindamycin effect is also dependent on exposure. However, its clinical efficacy is more closely related to the area under the concentration curve over the MIC for 24 hours ($fAUC_{0\text{-}24h}$ /MIC) rather than the time that the unbound serum concentration exceeds MIC[14].

As a final step, Monte Carlo Simulations (MCS) can be performed to evaluate differences in the efficacy of various regimens used to prevent GBS disease. In this context MCS is used to evaluate the probability of target attainment (PTA), using the derived pharmacokinetic parameters, data on the concentration-effect relationship and the inter-individual variability[15-17]. In this way, the pharmacokinetic data of both mother and child, combined with the knowledge on the concentration-effect relationship can be used to optimize the antibiotic prophylaxis in clinical practice.

The aim of the research project presented in this thesis was to describe the pharmacokinetics of the antibiotics frequently used during pregnancy and delivery to prevent GBS infection. Because amoxicillin is the antibiotic most frequently used in the Medical Center Haaglanden, amoxicillin has been used as prototype. Furthermore, limited data on the pharmacokinetics of clindamycin and penicillin G were described.

Pharmacokinetics of antibiotics in pregnancy and delivery: Amoxicillin as a prototype

Pregnant women with preterm premature rupture of the membranes (PPROM) are an important group of patients treated with amoxicillin. In these women, the mechanical barrier of the membranes is disturbed and it is believed that bacteria that are normally present in the lower genital tract might ascend to the uterus and

cause intrauterine infection. The immune system of premature neonates is not fully developed and therefore they are even more at risk for infection.

Estimates of the pharmacokinetic parameters of amoxicillin in women with PPROM did not differ significantly from values reported in the literature for non-pregnant individuals (**Chapter 4**). The only difference between the results of our study and previously reported values for non-pregnant individuals were the peak-concentrations. Peak-concentrations reached in pregnant women were lower compared to non-pregnant individuals. This might be explained by an increase of extracellular fluid or the presence of the fetus, placenta and amniotic fluid. There are previous studies on several drugs that do show differences in the pharmacokinetics between pregnant women and non-pregnant individuals[18,19]. Most of these studies included both patients before the onset of and during labor. Patients with PPROM were all included before the onset of labor. One study on the pharmacokinetics of ampicillin found that the terminal half-life was increased in women during labor when compared to pregnant women before the onset of labor[20]. Since the patients in our study were not in labor this could explain why our study did not show differences in pharmacokinetics between non-pregnant individuals and pregnant women. In other words, not the state of pregnancy, but being in labor might influence the pharmacokinetics. And indeed, for amoxicillin we found a minor influence of labor on the pharmacokinetics (**Chapter 5**). The volume of distribution of the peripheral compartment was decreased, but this did not result in a changed terminal half-life. Thus, although we did observe some slight differences, we do not consider them clinically relevant.

Another factor that might influence the pharmacokinetics of amoxicillin is the simultaneous administration of clavulanic acid. Potential interactions between simultaneously administered drugs might be caused by inhibition of metabolic enzymes, competition for protein binding in plasma or changes in renal transporter-system. When amoxicillin and clavulanic acid are administered simultaneously, the activity spectrum of amoxicillin against both gram-positive and gram-negative bacteria is enhanced. Amoxicillin is inactivated by β-lactamases produced by several microorganisms. When clavulanic acid is administered simultaneously it protects the amoxicillin from enzymatic inactivation[21] by irreversibly binding and inhibiting a wide range of these β-lactamases. The intrinsic antibacterial activity of clavulanic acid alone is negligible[21]. In practice different dose ratios of amoxicillin/clavulanic acid have been used. As there are no reports that the efficacy of the these dose ratios are different, the amount of clavulanic acid may not be that critical[22]. Time-concentration profiles of amoxicillin are therefore even more important. Clavulanic acid and amoxicillin have, in part, similar routes of metabolization and excretion. But, the pharmacokinetics of amoxicillin in maternal serum was found not to be influenced by simultaneous administration of clavulanic acid (**Chapter 6**).

In patients with a intra-amniotic infection (IAI) antibiotic treatment will be continued following delivery. In the immediate postpartum period, physiological changes in the mother start to take place. Such changes may have major influences on the pharmacokinetics as has previously been shown for the antiepileptic drug lamotrigine[23]. After delivery the concentration of lamotrigine rises to toxic levels. Therefore knowledge on the pharmacokinetics after delivery is needed. The peripheral volume of distribution of amoxicillin appeared to be decreased during labor, but decreases further immediately after delivery. A decrease in volume of distribution might result in higher concentrations in maternal serum, as has been shown for lamotrigine. However, for amoxicillin toxic concentrations are not reached and the current dosing regimens do not need to be adapted (**Chapter 5**).

For ampicillin/ amoxicillin, two different dosing regimens are described in the literature to prevent neonatal GBS disease. The Centers for Disease Control and Prevention (CDC) recommend an initial dose of 2 gram, and subsequent doses of 1 gram every 4 hours[2]. In the Cochrane Library a dosing regimen of 1 gram every 6 hours is described as the usual regimen[24]. Although the rate of infusion influences the PTA, infusion rates are not described in the current guidelines. Doses of 2 gram amoxicillin are administered by infusion over 30 minutes, whereas doses of 1 gram amoxicillin may be administered by infusion (i.e. over 15 minutes) or as bolus injection. However, our simulation studies suggest that the 2 gram starting dose does not have additional value over a 1 gram dose. In general, a slower infusion rate will increase the PTA and for that reason infusions are preferred over bolus injections. However, bolus injections are in clinical practice more convenient. In our simulation study, the %*f*T>MIC after a bolus injection was only 4% lower compared to an infusion over 15 minutes. Values for the %*f*T>MIC found for both regimens with the antibiotics administered by bolus injections reached adequate concentrations taking into consideration the mean population and the 99% confidential intervals. Therefore, we conclude that bolus injections can be used in these regimens without a reduction in the efficacy (**Chapter 8**).

To recommend a particular dosing regimen, several aspects should be taken into account. Since the initial dose of 2 gram does not have additional value over a 1 gram dose, the only difference between the two regimens is the dosing interval of 4 hours versus 6 hours. Although larger intervals reduce the %*f*T>MIC, both regimens reach adequate maternal concentration-time profiles. However, to guarantee that the efficacy to prevent neonatal GBS disease is not reduced using a 6-hour dosing interval, time-concentration profiles in the fetus should also be taken into account. To remain on the safe side, we recommend a dosing interval of 4 hours. Another argument to recommend a 4-hour dosing regimen instead of a 6-hour dosing interval is the clinical situation in which the prophylaxis has to be administered. The often urgency of care in delivery rooms can easily result in inaccuracies in the administration. Using a 4-hour dosing interval results in a higher

PTA, also when doses are accidentally skipped (**Chapter 8**).

So far, we conclude that the recommended dosing regimens reach adequate concentration-time profiles in maternal serum. Being in labor as well as the co-administration of clavulanic acid does not result in clinically relevant changes in the amoxicillin pharmacokinetics. Since the most important question is whether these regimens are also adequate to prevent neonatal GBS disease, we have also studied the transfer of amoxicillin over the placental barrier and the pharmacokinetics in the fetus.

Antibiotics administered to the mother reach the fetus via the umbilical cord. Venous cord blood reaches the fetus after placental exchange, whereas arterial umbilical cord blood originates from the fetal circulation. Drug concentrations in the arterial umbilical cord serum are therefore representative of the concentrations in the fetus. In the investigation described in **chapter 7** these concentrations have been analyzed together with samples from the neonates taken approximately 20 minutes after birth with a heel puncture. Specifically in this investigation data on the maternal pharmacokinetics, transfer over the placental barrier and pharmacokinetics in the fetus were all analyzed simultaneously in a 5-compartment model. This gave us the unique opportunity to study the different concentration-time profiles in the mother and the fetus in relation to the time. The peak-concentration in fetal serum was lower and delayed compared to the maternal peak-concentration. The peak-concentration in venous umbilical cord serum was lower compared to the maternal peak-concentration, but higher in comparison to the fetal peak-concentration. After similar values for venous umbilical cord and fetal serum were reached, the fetal concentrations exceeded the concentrations in umbilical cord. Considering the population estimates for the pharmacokinetics in the venous umbilical cord and the fetus, the initial infusion of 2 gram amoxicillin is adequate to prevent GBS infection in the fetus (**Chapter 7**). However, data on the inter-individual variability were not included and these results are therefore only valid for the average pregnant women with an average fetus. Therefore no final conclusion on the efficacy of amoxicillin in the prevention of neonatal GBS disease can be drawn from these data.

In clinical practice, the period between the start of the first antibiotic dose and birth is often used as measure of the adequacy of the prophylaxis. A period of less than 4 hours is considered as inadequate and antibiotic therapy is often continued in these neonates. In contrast, when neonates are born more than 4 hours after the start of the first dose, prophylaxis is considered adequate and they are usually not treated with antibiotics. This clinical measure of adequacy is based on two premises: first on the relation between the number of positive blood cultures and the time after a single antibiotic dose and secondly on the number of vertical transmissions from the maternal vagina to the neonatal mucocutaneous surfaces. Both the number of positive blood cultures and the number of vertical transmissions will decrease with time, because a minimum amount of time is needed to eradicate microorganisms.

After the peak-concentration in the fetus has been reached (31 minutes after the maternal peak-concentration) the concentration will decrease. Antibiotic present in the fetus will eradicate microorganisms and this eradication will occur independent from birth. Therefore, the current clinical measure of efficacy is not correlated to the true efficacy of the antibiotic prophylaxis (**Chapter 2 and 7**).

Patients treated with amoxicillin in our study groups were all relatively healthy. One patient was diagnosed with PPROM and suffered from severe vomiting during the first part of the study (**Chapter 9**). Her concentration-time profile during the first amoxicillin administration deviated substantially from the normal profile, but after she stopped vomiting the profile became comparable to the profiles described in chapter 4. As after both antibiotic administrations, the entire doses were absorbed in the body, improper infusion could not explain the difference between the two profiles. We hypothesized that several (patho)physiological changes had occurred, that all influenced the peripheral blood flow and thereby changed the distribution of the amoxicillin. As a possible explanation it was suggested that the arm in which the amoxicillin was infused might act as depot for the amoxicillin. After the blood flow slowly normalizes the amoxicillin is steadily released from the depot. The registration of this profile is unique because blood samples were taken frequently in an acute, emotional and very stressful situation. This indicates that although the pharmacokinetics is quite well described in healthy volunteers and several groups of patients, unexpected differences in the pharmacokinetic profile can occur in (critically) ill patients.

To conclude, for amoxicillin the concentration-time profiles in pregnant patients before the onset of labor, during labor and in the immediate postpartum period were such that the currently advised dosing regimens in the prevention of GBS disease may be considered adequate. The results of the MCS showed that even when a dose is accidentally skipped, the percentage of time the concentration exceeded the MIC for GBS still reached the threshold value. The pharmacokinetic profile for the average pregnant women and the predicted concentration-time profile in the average fetus indicated that the dosing regimen is also adequate in preventing neonatal GBS disease, albeit that in this prediction the inter-individual variability could not be taken into account.

Clindamycin

Clindamycin is often used as an alternative to amoxicillin in the prevention of GBS infections[2]. The efficacy of the clindamycin dosing regimens in this indication should be studied in a similar manner as performed for amoxicillin. However, to date only a limited number of patients treated with clindamycin could be included

in the study. Therefore no final conclusions can be drawn from this study (**Chapter 10**).

With regard to the pharmacokinetics of clindamycin plasma protein binding is a critical factor as it is an important determinant of efficacy. As clindamycin binds largely to alpha-1-acid glycoprotein wide variations in the degree of protein binding may be expected. Most values on the plasma protein binding of clindamycine reported in the literature range between 80-90%[25-27]. The percentage might be decreased in pregnant women, but in patients with an infection or stress it is shown to be increased. Therefore, the protein binding in our patients at risk is not likely to be less than 80%. For the average pregnant women the currently used dosing regimen leads to adequate concentrations assuming that the protein binding will not exceed 80% of the total concentration. However, to recommend a dosing regimen for the entire population of pregnant women, an adequate concentration-time profile for the average women is not sufficient and the inter-individual variability has to be taken into account. When taking into account the 99% confidence intervals, this regimen was shown not to be adequate when protein binding exceeds the value of 80% (**Chapter 10**).

There were insufficient data of the transfer of clindamycin over the placental barrier to incorporate these data in the pharmacokinetic model. Therefore the individual concentrations in umbilical cord serum are presented which ranged from 0.1 mg/L to 4 mg/L. Considering the protein binding, dosing interval of 8 hours and the MIC value for GBS (0.5 mg/L)[28], these data indicate that with the current dosing regimen the exposure of the fetus to clindamycine may be inadequate for the prevention of neonatal GBS disease.

It has been demonstrated that not all women with a penicillin-allergy in their history are actually allergic to penicillins[29,30]. It may therefore be useful to re-evaluate the hypersensitivity to penicillin. It has previously been shown that allergy testing is safe during pregnancy and that penicillins can be used in women with a negative allergy skin test[31]. Until further studies performed demonstrating adequate pharmacokinetics of clindamycin in pregnancy and especially in the arterial umbilical cord or fetus for the prevention of GBS disease in the mother and the infant, allergy skin testing can be performed to avoid the use of clindamycin.

Treatment with penicillin G in premature neonates

In neonates with suspected GBS infection, the administration of antibiotics to the neonate will be started immediately after birth. For several drugs it has been demonstrated that the pharmacokinetics in neonates differs substantially from that in adults[32-34]. Especially in premature neonates the terminal half-life is often prolonged. Similar to this, we found a prolonged terminal half-life of penicillin G

of 3.9 h in neonates with a gestational age of less than 32 weeks on the third day of life. Taking into account the inter-individual variability, the currently used dosing regimen of 50.000 U/kg every 12 h was shown to be adequate for the treatment of neonatal infections caused by common microorganisms on day 3 of life (**Chapter 11**).

The prolonged half-life in (premature) neonates is particularly beneficial for antibiotics with a time-dependent mechanism of action, since the time the concentration remains above the MIC will be increased. The prolonged half-life is most likely caused by immature renal function. This supports our opinion that the clinically used measure of efficacy (i.e. a period of 4 hour between the start of the antibiotic administration and birth) may need to be reconsidered. After the peak concentration in fetal serum has been reached the concentration will decrease. Since the terminal half-life in the fetus is likely to be lower compared to the neonatal half-life, the highest values for the T>MIC will be reached when birth takes place soon after the peak concentration has been reached.

Future perspectives

The frequency of the use of antibiotics in the peripartum period to prevent GBS disease has increased the last decades. This is the result of the change in prevention strategy to a screening-based strategy and an increase in the prevalence of GBS in pregnant women. In the near future more sensitive detection methods are likely to become available. As a result also women with a low number of GBS in their rectovaginal tract will be identified as GBS carrier. It is doubtful whether these women indeed have an increased risk on giving birth to a neonate with GBS disease. Nevertheless since increasing percentages of pregnant women will be candidates for antibiotic prophylaxis, it is important that the dosing regimen is adequate for the entire population. Suboptimal concentrations pose patients at risk for failure of prophylaxis and might cause selection of resistant bacterial strains.

From the data described in this thesis, it is likely that for amoxicillin current dosing regimens are adequate for healthy women, also when minor dosing inaccuracies occur. However, to guarantee that the concentrations reached in the fetus are also adequate, Monte Carlo Simulations should be performed using the 5-compartment model for the simultaneous analysis of the concentration-time profiles in the mother and the fetus, taking into the account the inter-individual variability. In this way, an optimal dosing regimen can be chosen for the entire population. Unfortunately, there are no computer programs available to perform MCS with a 5-compartment model.

Patients treated with amoxicillin included in our study were all healthy, with one exception. This patient was included while she suffered from unexplained

severe vomiting. The pharmacokinetic profile in this patient was dramatically different from profiles in relatively healthy patients. This indicates that physiological changes might have major influences on the pharmacokinetics. In our study group it was found that several physiological factors had an influence on the amoxicillin pharmacokinetics. For example, the amount of edema influenced the volume of distribution. Pregnancy and delivery are sometimes complicated by particular disorders. One of these pregnancy related disorders, preeclampsia, is reknowned for an increased amount of edema. This can add up to 10 liter of extracellular fluid. One can therefore not assume that the concentration-time profiles in women with preeclampsia are also adequate. Therefore, pharmacokinetic studies in patients with pregnancy-related disorders should be performed.

For clindamycin limited pharmacokinetic data are available. Considering high plasma protein binding for clindamycin, it appears that the concentration-time profiles may be inadequate for the prophylaxis of GBS infections in both the mother and the fetus. Only the unbound fraction of the drug is active and crosses the placental barrier and therefore changes in protein binding might influence the efficacy of the clindamycin. It is known that protein binding of clindamycin is dependent both on the serum concentration of alpha1-acid glycoprotein, which changes in pregnant women, and on the serum clindamycin concentration. Unfortunately, relatively large blood samples are needed to determine the protein binding in serum. The volume of the blood samples obtained in our study was too small to determine the protein binding. Patients with chronic hart conditions were included in the study. The pharmacokinetics were found to be unaltered in this patient group. Future studies are needed to prove the efficacy of the current dosing regimen and they should include patients with pregnancy-related disorders and information on the protein binding. Finally, a pharmacokinetic model should be developed based on pharmacokinetic parameter estimates of both the mother and fetus, the inter-individual variability as well as data on the clindamycin protein binding to investigate the predicted efficacy of the different dosing regimens using Monte Carlo Simulation.

In the US, *Eschericia coli* is becoming more common as a cause of neonatal infection, especially in premature neonates[35,36]. Many strains of *E. coli* are resistant to amoxicillin and therefore the use of amoxicillin/clavulanic acid might become more commonly used in the future. We showed that the pharmacokinetics of amoxicillin in pregnant women is not influenced by the simultaneous administration of clavulanic acid. However, for inactivation of β-lactamases by clavulanic acid, there has to be a minimal amount of clavulanic acid present. Therefore, future studies should be performed on the pharmacokinetics of clavulanic acid. This harbors a practical problem. Clavulanic acid is a very unstable compound. Therefore, concentrations of clavulanic acid should preferably be determined immediately after the samples are obtained from the patients and a special HPLC method should be developed to

make sure that the clavulanic acid is not degradated during the procedure. Finally, the development of methods for the stabilization in clavulanic acid in plasma should be explored.

In conclusion, the present study indicates that the currently advised dosing regimen for amoxicillin is effective to prevent neonatal GBS disease. But research on the pharmacokinetics of various antibiotics should be continued for optimization of the GBS disease prophylaxis. Especially, the development of pharmacokinetic models describing the maternal and fetal pharmacokinetics simultaneously as well as their relation in time, taking into account the inter-individual variability and degree of protein binding is of importance. Patients with pregnancy-related disorders should be included in these studies. Also for the currently recommended dosing regimen for clindamycin, the results of current study raise some doubt on the efficacy to prevent neonatal GBS disease. The antibiotics of first choice remain the penicillins. When penicillin allergy is encountered, skin testing is advised to ensure that the use of amoxicillin is contraindicated.

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Samenvatting in het Nederlands

Populatie farmacokinetiek van antibiotica ter preventie van groep B streptokokken-ziekte:

Samenvatting, conclusies en toekomstperspectieven.

Afkortingen

AUC	Area under the curve - oppervlak onder de grafiek
CDC	Centers for Disease Control and Prevention
GBS	Groep B streptokokken
MCS	Monte Carlo Simulaties
MRC	Minimaal remmende concentratie
PPROM	Preterm Premature Rupture of the Membranes - prematuur preterm gebroken vliezen

Introductie

Groep B streptokokken (GBS) zijn belangrijke veroorzakers van infectie gerelateerde morbiditeit en mortaliteit rondom de bevalling. Vanaf 1970 zijn GBS-gerelateerde infecties in opmars en ze werden zo de meest frequente oorzaak van neonatale morbiditeit en mortaliteit. GBS-ziekte komt bij neonaten meestal tot uiting als pneumonie, sepsis of een meningitis. Het is minder bekend dat GBS rondom de bevalling ook verschillende infecties bij de moeder kan veroorzaken. Cervicovaginale kolonisatie is meestal asymptomatisch, maar GBS kan ook urineweginfecties, vulvovaginitis, intra-uteriene infectie, mastitis, bacteriemie, sepsis, meningitis, endometritis en wondinfecties veroorzaken. Deze infecties hebben vaak niet alleen consequenties voor de moeder, maar ook voor het kind (beschreven in **Hoofdstuk 3**).

Sinds de jaren negentig zijn in verschillende landen maatregelen getroffen om neonatale GBS-ziekte te voorkomen. Het preventief toedienen van antibiotica aan de moeder kort voor of tijdens de bevalling is hierbij het belangrijkste onderdeel. Om het gebruik van deze antibiotica te kunnen aanbevelen is het noodzakelijk de positieve effecten en de bijwerkingen tegen elkaar af te wegen. Globaal genomen is het voorkomen van bewezen vroege neonatale GBS-ziekte (GBS-ziekte in de eerste 7 dagen na de geboorte bewezen met een kweek) met introductie van deze maatregel gedaald. Dit wekt de indruk dat de antibiotica effectief zijn. Deze incidentie cijfers worden echter beïnvloed door meerdere factoren. Daarnaast zijn er gevallen van neonatale GBS-ziekte beschreven bij kinderen van wie de moeder tijdens de bevalling was behandeld met antibiotica. Hierdoor ontstaat twijfel over de effectiviteit van de profylaxe. Een specifieke vraag hierbij is of in de huidige richtlijnen de optimale dosering en het optimale doseringsinterval wordt gehanteerd. Bijwerkingen van antibiotica bij de moeder, zoals een anafylactische reactie, komen zelden voor, maar zullen bij sterk toenemend gebruik vaker gezien worden. Tevens is er toenemende bezorgdheid over mogelijke negatieve effecten voor het kind. Het gebruik van antibiotica tijdens de bevalling heeft mogelijk effect op de samenstelling van de initiële bacteriële flora van het kind. Een veranderde bacteriële flora zou een effect kunnen hebben op de ontwikkeling van het immuunsysteem van de neonaat. Terwijl er matig bewijs is voor de effectiviteit van de antibiotica en weinig aandacht voor de bijwerkingen, wordt in de richtlijnen van de Nederlandse Vereniging van Obstetrie en Gynaecologie en de Centers for Disease Control and Prevention het gebruik van antibiotica rondom de bevalling geadviseerd aan hoge percentages zwangere vrouwen (tot 35% van alle zwangere vrouwen) (beschreven in **Hoofdstuk 2**).

Om onderzoek te doen naar de effectiviteit van antibiotica en om het optimale doseringschema ter preventie van GBS-ziekte te bepalen, is kennis over de farmacokinetiek noodzakelijk. Farmacokinetiek is het vakgebied dat wiskundige

modellen toepast om het verloop van de concentraties van geneesmiddelen in het lichaam te beschrijven en te voorspellen. Het verloop van de concentratie van het geneesmiddel in de tijd wordt bepaald door de mate en snelheid van absorptie, distributie, metabolisatie en excretie.

Farmacokinetische studies in de zwangerschap: algemene benadering

Antibiotica die gebruikt worden ter preventie van GBS-ziekte worden toegediend aan de moeder, terwijl deze tevens bedoeld zijn om de foetus te beschermen. Antibiotica in de foetale circulatie zijn afkomstig van de maternale circulatie na passage van de placenta. Het is hierom essentieel voor de foetus dat het concentratieverloop in de moeder adequaat is. Bij het bestuderen van de farmacokinetiek ten behoeve van de profylaxe tegen GBS-ziekte dient het concentratie-tijd verloop in de moeder dus als eerste bestudeerd te worden. Tijdens de zwangerschap zijn er veranderingen in de absorptie, distributie, metabolisatie en excretie van geneesmiddelen. Zo neemt bijvoorbeeld het plasma in volume toe, terwijl de binding aan albumine afneemt. Ook de bloedstroom naar de lever, evenals de glomerulaire filtratiesnelheid in de nier neemt toe. Omdat al deze veranderingen tegelijkertijd optreden en tevens afhankelijk zijn van de zwangerschapsduur, is het niet op voorhand duidelijk hoe de farmacokinetiek geëxtrapoleerd moeten worden uitgaande van gegevens verkregen in niet-zwangere personen.

Wanneer vastgesteld is dat het toegediende antibioticum bij de moeder een adequaat concentratie-tijd verloop heeft, dan is als tweede de passage van het antibioticum door de placenta van belang. Zo bereikt het antibioticum immers de foetus. De snelheid waarmee het antibioticum over de placenta wordt getransporteerd is afhankelijk van de maternale-foetale concentratie gradiënt en omgekeerd evenredig met de doorlaatbaarheid van het membraan van de placenta. De dikte van de placenta neemt toe met de zwangerschapsduur maar ook bij een aantal aandoeningen die de zwangerschap kunnen compliceren, zoals diabetes mellitus en zwangerschapsvergiftiging. Concentraties die gemeten worden in bloedmonsters van het veneuze navelstrengbloed kunnen informatie geven over het transport van het antibioticum over de placenta. Helaas kunnen bloedmonsters van de navelstreng na de bevalling slechts één keer per patiënt afgenomen worden en het moment van afname kan niet door de onderzoeker gepland worden.

Ten slotte dient de farmacokinetiek van de antibiotica in het kind bestudeerd te worden. Dit kan gedeeltelijk worden gebaseerd op concentraties in arteriële bloedmonsters afkomstig uit de navelstreng direct na de geboorte, maar ook met bloedmonsters van de neonaat. Hierbij is het belangrijk te onderkennen dat er farmacokinetische verschillen zijn tussen de foetus en de neonaat. Voor de

geboorte is de bloedstroom terug naar de moeder de voornaamste route van excretie. Echter na de geboorte is de navelstreng afgeklemd en vindt de excretie plaats via de organen van de neonaat. Daarnaast zal ook het bloed zich na de geboorte anders over het lichaam verdelen dan voor de geboorte. Ook dit zou de farmacokinetiek kunnen beïnvloeden.

Samenvattend kan gesteld worden dat het nodig is om allereerst de farmacokinetiek in zowel de moeder als het kind te bestuderen bij onderzoek naar de effectiviteit van antibiotica die gebruikt worden als profylaxe tegen GBS-ziekte. In tegenstelling tot maternale bloedmonsters, die relatief vaak afgenomen kunnen worden, kunnen na de geboorte bloedmonsters van de navelstreng slechts één keer per patiënt afgenomen worden. Dit zal resulteren in groepen van ongelijke grootte. Gelukkig, kunnen data tegenwoordig geanalyseerd worden met de zogenaamde "populatie benadering" met computerprogramma's zoals NONMEM (Non-linear Mixed Effects Modeling). Bij deze benadering worden de data van alle individuen in de populatie tegelijkertijd geanalyseerd, waarbij rekening gehouden wordt met de inter-individuele en intra-individuele variabiliteit. Ook kunnen gegevens van groepen met verschillende grootte evenals gegevens van patiënten waarvan onvolledige gegevens beschikbaar zijn, met deze methode worden geanalyseerd.

Om de profylaxe te kunnen evalueren is naast kennis van de farmacokinetiek ook inzicht in de relatie tussen de farmacokinetische eigenschappen en het klinisch effect van groot belang. Tijdens de zwangerschap en bevalling worden verschillende antibiotica gebruikt. In Europa wordt amoxicilline vaak gebruikt ter preventie van GBS ziekte. In sommige ziekenhuizen wordt amoxicilline gecombineerd met clavulaanzuur, omdat deze combinatie ook werkzaam is tegen amoxicilline-resistente Escherichia coli. In geval van allergie voor penicillines wordt clindamycine voorgeschreven als alternatief. De relatie tussen de farmacokinetische eigenschappen, de gevoeligheid van het micro-organisme (als weerspiegeld door de minimaal remmende concentratie, MRC) en de klinische effecten wordt steeds beter begrepen. De effectiviteit van de penicillines, zoals amoxicilline, is gecorreleerd met het percentage van de tijd dat de serum concentratie van het ongebonden geneesmiddel hoger is dan de MRC (%*f*T>MRC). Het effect van clindamycine is, net zoals dat van amoxicilline, afhankelijk van de blootstelling, maar de klinische effectiviteit wordt voor dit antibioticum het best beschreven door het oppervlak onder de grafiek (area under the curve, AUC) boven de MRC gedurende 24 uur (*f*$AUC_{0\text{-}24h}$/MRC).

Met behulp van Monte Carlo Simulaties (MCS) kunnen verschillen in effectiviteit tussen verschillende doseringschema's voor de preventie van GBS ziekte worden geëvalueerd. Bij deze simulaties wordt gebruik gemaakt van de waarden van de farmacokinetische parameters, de relatie tussen de concentratie van het antibioticum en het effect alsmede de inter-individuele variabiliteit. Zo kan een aantal scenario’s worden geëvalueerd. Op deze manier kan de farmacokinetische

data van zowel moeder als kind worden gecombineerd met de kennis over de concentratie-effect relatie om de antibioticum profylaxe in de klinische praktijk te optimaliseren.

Het doel van het onderzoeksproject gepresenteerd in dit proefschrift was om de farmacokinetiek van antibiotica die veelvuldig gebruikt worden tijdens de zwangerschap en bevalling ter preventie van GBS ziekte te beschrijven. In het Medisch Centrum Haaglanden wordt meestal amoxicilline gebruikt. Om deze reden is amoxicilline in dit proefschrift als prototype gebruikt. Daarnaast werden ook in beperktere mate onderzoek verricht naar clindamycine en penicilline G.

De farmacokinetiek van antibiotica tijdens de zwangerschap en bevalling: Amoxicilline als prototype.

Zwangere vrouwen met prematuur preterm gebroken vliezen (PPROM) vormen een belangrijke groep van patiënten die met amoxicilline wordt behandeld. Bij deze vrouwen is de mechanische barrière tegen infectie die gevormd wordt door de vliezen verstoord en men neemt aan dat de normale genitale flora de uterus kan binnendringen, waarna een intra-uteriene infectie kan ontstaan. Het immuun systeem van premature neonaten is bovendien nog niet volledig ontwikkeld en dat maakt juist deze groep extra gevoelig voor infectie.

De schattingen voor de farmacokinetische parameters van amoxicilline bij vrouwen met PPROM waren niet anders dan de waarden die eerder in de literatuur zijn beschreven voor niet-zwangere individuen (**hoofdstuk 4**). Alleen de piek-concentraties in onze groep patiënten waren lager dan bij niet-zwangeren. Mogelijk is dit te verklaren door de toegenomen hoeveelheid extracellulaire vloeistof of de aanwezigheid van de foetus, placenta en vruchtwater. In de literatuur zijn voor een aantal andere geneesmiddelen wel verschillen gevonden in de farmacokinetiek tussen zwangeren en niet-zwangeren. In de meeste van deze studies werden zowel vrouwen voor de bevalling als tijdens de bevalling geïncludeerd. Bij patiënten met PPROM is de bevalling nog niet op gang gekomen. Een studie naar de farmacokinetiek van ampicilline toonde aan dat er verschillen in de farmacokinetiek waren tussen zwangere vrouwen voor de bevalling en tijdens de bevalling, terwijl er geen verschillen tussen zwangere vrouwen en niet-zwangere vrouwen werden gevonden. Het zou dus zo kunnen zijn dat de veranderingen die optreden tijdens de bevalling zorgen voor verschillen in farmacokinetiek tussen de groepen en niet de zwangerschap op zich. Net zoals bij deze studie met ampicilline vonden wij inderdaad een kleine invloed van de bevalling op de farmacokinetiek van amoxicilline (**hoofdstuk 5**). De grootte van het verdelingsvolume was afgenomen tijdens de bevalling. Deze verandering was echter zo klein dat het geen invloed heeft

op de halfwaarde tijd. De verandering in farmacokinetiek is daardoor waarschijnlijk niet klinisch relevant.

Een andere factor die invloed zou kunnen hebben op de farmacokinetiek van amoxicilline is de gelijktijdige toediening van clavulaanzuur. Eventuele interacties tussen twee geneesmiddelen kunnen veroorzaakt worden door remming van metabole enzymen, competitie voor eiwit binding in het plasma of veranderingen in het transporter-systeem van de nier. De gelijktijdige toediening van amoxicilline en clavulaanzuur vergroot het antibacteriële spectrum tegen gram-positieve en gram-negatieve bacteriën. Amoxicilline kan namelijk worden geïnactiveerd door β-lactamases, enzymen die door verschillende micro-organismen geproduceerd worden. Wanneer clavulaanzuur toegevoegd wordt aan de amoxicilline beschermt het clavulaanzuur amoxicilline tegen enzymatische inactivatie door irreversibel te binden aan deze β-lactamases. Clavulaanzuur zelf heeft nauwelijks een antibacterieel effect. In de loop der jaren is de verhouding in de hoeveelheid amoxicilline en clavulaanzuur gewijzigd, maar dit lijkt geen invloed te hebben op de effectiviteit. Waarschijnlijk is de hoeveelheid clavulaanzuur dus minder van belang dan de hoeveelheid amoxicilline. Ondanks dat clavulaanzuur gedeeltelijk via dezelfde routes van metabolisatie en excretie wordt geëlimineerd als amoxicilline, wordt de farmacokinetiek tijdens de zwangerschap niet beïnvloed door de toevoeging van clavulaanzuur (**hoofdstuk 6**).

Voortzetten van de antibiotische behandeling onmiddellijk na de bevalling wordt geadviseerd bij vrouwen bij wie een intra-uteriene infectie wordt vermoed. Direct na de bevalling beginnen er fysiologische veranderingen op te treden. Deze veranderingen in de fysiologie kunnen leiden tot relevante veranderingen in de farmacokinetiek. Een goed voorbeeld hiervan is dat bij vrouwen met epilepsie de concentratie lamotrigine kort na de bevalling kan stijgen tot toxische waarden. Dit voorbeeld laat zien hoe belangrijk het kan zijn om veranderingen in de farmacokinetiek te bestuderen. Voor amoxicilline is het verdelingsvolume direct na de bevalling kleiner geworden. Net zoals voor lamotrigine zou dit kunnen leiden tot hogere concentraties. Met het huidige doseringschema worden toxische concentraties echter niet bereikt en daardoor zijn aanpassingen in de dosering direct na de geboorte niet nodig (**Hoofdstuk 5**).

In de literatuur zijn voor ampicilline en amoxicilline twee verschillende doseringschema's beschreven ter preventie van GBS ziekte. Het 'Centers for Disease Control and Prevention' (CDC) adviseert een initiële dosis van 2 gram en vervolgens elke 4 uur een dosis van 1 gram. De 'Cochrane Library' beschrijft echter het doseringschema van 1 gram elke 6 uur als het meest gebruikte. Een belangrijke vraag is of er verschillen zijn in de effectiviteit van deze behandelschema's. Zoals hierboven vermeld kan deze vraag worden beantwoord op basis van de uitkomsten van Monte Carlo Simulaties. Ondanks het feit dat de toedienings-snelheid van de antibiotica invloed kan hebben op de effectiviteit, wordt dit nergens beschreven in

de richtlijnen. Een dosis van 2 gram amoxicilline wordt toegediend in een infuus van 30 minuten, terwijl de dosis van 1 gram gegeven kan worden als infuus van 15 minuten of als bolus injectie. De resultaten van Monte Carlo Simulaties van deze verschillende doseringen laten zien dat het geven van een initiële dosis van 2 gram waarschijnlijk geen toegevoegde waarde heeft boven de dosis van 1 gram. In principe kan een langzamere infusie de waarschijnlijkheid op het behalen van het therapeutische doel vergroten. Hierdoor worden deze verkozen boven de bolus injecties, die in het dagelijkse gebruik eenvoudiger zijn. Onze studie laat zien dat het percentage van de tijd dat de ongebonden concentratie boven de MRC (%*f*T>MRC) is, inderdaad hoger is bij een infusie dan bij de bolus injectie. Het verschil in %*f*T>MRC tussen de infusie van 15 minuten en de bolus injectie is echter slechts 4%. Bij simulatie van de in de literatuur beschreven doseringsschema's met bolus injecties van 1 gram, bleek dat de verkregen concentratie-tijd profielen voor de populatie rekening houdend met het 99% betrouwbaarheidsinterval adequaat waren. Hieruit concluderen we dat bij de dosering van ampicilline en amoxicilline bolus injecties gebruikt kunnen worden zonder dat de effectiviteit van de profylaxe minder wordt (**Hoofdstuk 8**).

Om een bepaald doseringsschema aan te bevelen, moeten een aantal aspecten in acht worden genomen. We hebben eerder al laten zien dat de initiële dosis van 2 gram geen toegevoegde waarde heeft boven de dosis van 1 gram. Het enige verschil tussen de beschreven doseringsschema's is dan het doseringsinterval van 4 uur versus 6 uur. Ondanks dat het verlengen van het doseringsinterval de %*f*T>MRC verlaagt, worden bij de moeder adequate concentraties bereikt bij beide doseringsschema's. Echter, om zeker te zijn dat de effectiviteit van de profylaxe in het voorkomen van neonatale GBS-ziekte niet is afgenomen wanneer gebruik wordt gemaakt van een doseringsschema met een interval van 6 uur, moet ook rekening gehouden worden met het concentratie-tijd profiel in de foetus. Daarom raden we voor de zekerheid een doseringsinterval van 4 uur aan. Dit interval wordt ook aangeraden gezien de klinische situatie waarin de profylaxe wordt toegediend. De vaak hectische situatie in de verloskamer kan gemakkelijk leiden tot onnauwkeurigheden in de toediening. Wanneer gebruik wordt gemaakt van het doseringsinterval van 4 uur leiden onnauwkeurigheden in de toediening minder snel tot inadequate concentraties, zelfs wanneer per ongeluk een dosis wordt overgeslagen (**hoofstuk 8**).

Tot nu toe kunnen we concluderen dat de aanbevolen doseringsschema's bij de moeder adequaat zijn. Zowel de bevalling als het tegelijkertijd toedienen van clavulaanzuur leiden niet tot klinisch relevante veranderingen in de farmacokinetiek van amoxicilline. Maar omdat de belangrijkste vraag is of ook GBS-ziekte bij het kind voorkomen wordt, is ook onderzoek gedaan naar het transport van antibiotica over de placenta en naar de farmacokinetiek bij de foetus.

Antibiotica die toegediend worden aan de moeder bereiken de foetus via

de navelstreng. Het veneuze bloed in de navelstreng stroomt naar de foetus nadat het de placenta gepasseerd heeft, terwijl het arteriële bloed in de navelstreng direct afkomstig is uit de foetale circulatie. De antibioticum concentratie in het arteriële bloed in de navelstreng is daarom representatief voor concentraties in de foetale circulatie. In de studie beschreven in **hoofdstuk 7** worden deze concentraties geanalyseerd samen met concentraties in bloed dat werd afgenomen door middel van een hielprik bij de neonaten ongeveer 20 minuten na de geboorte. Bij deze studie werden gegevens over de farmacokinetiek bij de moeder, het transport over de placenta en de farmacokinetiek bij de foetus allemaal tegelijkertijd geanalyseerd door gebruik te maken van een 5-compartimenten model. Hierdoor was het mogelijk om de concentratie-tijd profielen van de moeder en de foetus met elkaar te vergelijken. Deze analyse laat zien dat de piek concentratie die in het foetale bloed wordt bereikt, lager en vertraagd is ten opzichte van de piek concentratie in het maternale bloed. De piek concentratie in het veneuze bloed van de navelstreng was lager dan die van de moeder, maar hoger dan die van de foetus. Op een bepaald moment worden de concentraties in het veneuze bloed van de navelstreng en in het foetale bloed gelijk, en daarna worden in het bloed van de foetus hogere concentraties bereikt dan in het veneuze bloed van de navelstreng. Wanneer gebruik wordt gemaakt van de verkregen populatie farmacokinetische schattingen voor de farmacokinetiek van amoxicilline in het veneuze bloed van de navelstreng en het bloed van de foetus, lijkt de initiële dosis van 2 gram adequaat voor het voorkomen van neonatale GBS-ziekte (**Hoofdstuk 7**). Echter, hierbij zijn gegevens over de inter-individuele variabiliteit nog niet meegenomen. Daarom gelden deze gegevens alleen voor de gemiddelde zwangere vrouw met een gemiddeld kind. Een definitieve conclusie die geldt voor de gehele populatie kan hieruit daarom niet worden getrokken.

In de praktijk wordt voor het bepalen van de effectiviteit van de profylaxe vaak gebruik gemaakt van de duur tussen de start van de toediening van de amoxicilline aan de moeder en de geboorte. Wanneer deze periode minder dan 4 uur is, wordt de profylaxe vaak als inadequaat beschouwd en wordt begonnen met antibiotische therapie bij de neonaat. Wanneer echter deze periode langer dan 4 uur is, wordt de profylaxe als adequaat beschouwd en ontvangt het kind doorgaans verder geen antibiotica. Deze klinische maatstaf voor effectiviteit is gebaseerd op twee waarnemingen. Ten eerste neemt het aantal positieve bloedkweken af bij het groter worden van het tijdsinterval tussen het toedienen van een enkele dosis antibioticum en het afname tijdstip van de bloedkweek.Ten tweede neemt het aantal transmissies van de maternale vagina naar de mucocutane oppervlakte van de neonaat af in de tijd. Een aannemelijke verklaring is dat er altijd een minimale tijdsduur nodig is om bacteriën te doden. Nadat de hoogste concentratie in het foetale bloed bereikt is (31 minuten na de hoogste concentratie bij de moeder), zal de concentratie dalen. De antibiotica in de foetale circulatie zullen micro-organismen doden onafhankelijk van

de geboorte. Hierdoor is de klinische maatstaf niet gecorreleerd met de werkelijke effectiviteit van de profylaxe (**hoofdstuk 2 en 7**).

Patiënten in onze studie zijn allemaal relatief gezond. Eén patiënt die opgenomen werd met PPROM braakte zeer hevig gedurende het eerste gedeelte van de studie (**Hoofdstuk 9**). Het concentratie-tijd profiel dat bij haar werd gemeten tijdens de eerste antibioticum toediening was afwijkend van het profiel bij gezonde patiënten. Nadat het braken was gestopt, was het concentratie-tijd profiel bij haar vergelijkbaar met dat van andere patiënten met PPROM (zoals beschreven in **Hoofdstuk 4**). Aangezien na beide toedieningen de totale doses geabsorbeerd waren door het lichaam, kon het verschil tussen de twee profielen niet verklaard worden door fouten in de toediening. Onze hypothese is dat een aantal (patho)fysiologische veranderingen die optraden, invloed hebben gehad op de perifere bloedstroom. Hierdoor veranderde de distributie van de amoxicilline in het lichaam. Onze hypothese is dat de arm waarin de amoxicilline toegediend was als een depot gefunctioneerd heeft. Nadat de bloedstroom langzaam normaliseerde, werd de amoxicilline alsnog langzaam over het lichaam verdeeld. Het vastleggen van een dergelijk concentratie-tijd profiel is uniek, aangezien bloedafname frequent plaatsvond in een acute, emotionele en zeer stressvolle situatie. Het concentratie-tijd profiel bij deze patiënte geeft aan dat ondanks dat de farmacokinetiek redelijk goed beschreven is bij gezonde vrijwilligers, er onverwachte veranderingen in de farmacokinetiek kunnen optreden bij (ernstig) zieke patiënten.

Samenvattend kan gezegd worden dat de concentratie-tijd profielen van amoxicilline bij zwangere vrouwen voor het begin van de bevalling, tijdens de bevalling en direct na de bevalling met de momenteel geadviseerde doseringsschema's adequaat zijn. MCS laten zien dat ook wanneer een dosis overgeslagen wordt, de tijd dat de ongebonden fractie amoxicilline hoger is dan de MRC nog steeds voldoende is. Voor de gemiddelde zwangere vrouw en haar gemiddelde kind is deze dosering eveneens adequaat om GBS-ziekte bij het kind te voorkomen. Gegevens van de inter-individuele variabiliteit konden in deze analyse helaas niet meegenomen worden.

Clindamycine

Ook clindamycine is een veelvuldig gebruikt antibioticum bij de preventie van GBS infecties. De effectiviteit hiervan voor deze indicatie dient op dezelfde wijze bestudeerd te worden als beschreven voor amoxicilline. Helaas kon slechts een beperkt aantal patiënten dat behandeld moest worden met clindamycine in de studie worden geïncludeerd. Hierdoor kunnen uit deze studie nog geen definitieve conclusies getrokken worden (**Hoofdstuk 10**).

Bij het bepalen van de effectiviteit van clindamycine is de eiwitbinding in het plasma van groot belang. Clindamycine bindt voornamelijk aan het acute fase eiwit alfa1-zure glycoproteine en het percentage eiwitbinding in het plasma varieert daardoor aanzienlijk. Het meest frequent worden echter waarden gerapporteerd tussen de 80% en 90%. Dit percentage neemt waarschijnlijk af tijdens de zwangerschap, maar neemt toe bij patiënten met een acute infectie of stress. Daarom is het aannemelijk dat het percentage eiwitbinding in onze patiënten ten minste 80% is. Voor de gemiddelde zwangere vrouw leidt het huidige doseringsschema tot adequate concentraties mits het percentage eiwitbinding niet hoger is dan 80%. Maar om een doseringsschema te adviseren dat adequaat is voor de gehele populatie is dit onvoldoende en dient tevens de inter-individuele variabiliteit in de resultaten verwerkt te worden. Uit de huidige resultaten kan niet geconcludeerd worden dat het toegepaste doseringsschema adequaat is voor de gehele populatie, vooral niet wanneer de eiwitbinding hoger is dan 80% (**Hoofdstuk 10**).

Om gegevens van de passage van clindamycine door de placenta in het model op te nemen, zijn meer gegevens nodig. Individuele concentraties in het bloed afkomstig uit de navelstreng variëren van 0.1 mg/L tot 4 mg/L. Wanneer rekening wordt gehouden met de eiwitbinding, een doseringsinterval van 8 uur en de MRC voor GBS (0.5 mg/L), geven deze data aan dat de concentraties in de foetus mogelijk inadequaat zijn voor de preventie van neonatale GBS-ziekte.

Het is bekend dat niet alle vrouwen die aangeven allergisch te zijn voor penicilline dit ook daadwerkelijk zijn. Het kan nuttig zijn om de allergie te herevalueren. Allergietesten kunnen uitgevoerd worden tijdens de zwangerschap en vrouwen waarbij de test negatief is kunnen met penicilline behandeld worden. Totdat meer onderzoek verricht is naar de effectiviteit van clindamycine ter preventie van neonatale GBS-ziekte, wordt een allergietest aanbevolen om het gebruik van clindamycine zo veel mogelijk te vermijden.

Behandeling met penicilline G van premature neonaten.

Bij neonaten bij wie een GBS infectie wordt vermoed zal direct na de geboorte met antibiotische behandeling begonnen worden. Van een aantal geneesmiddelen is bekend dat de farmacokinetiek in neonaten significant anders is dan in volwassenen. Vooral bij premature neonaten is de halfwaardetijd vaak verlengd. Ook wij vonden op de derde dag na de geboorte een langere halfwaardetijd van penicilline G (3.9 uur) bij neonaten geboren na een zwangerschapsduur van minder dan 32 weken. Wanneer rekening gehouden wordt met de inter-individuele variabiliteit, bleek dat het momenteel gebruikte doseringsschema van 50.000 eenheden/kg elke 12 uur adequaat is voor de behandeling van neonatale infecties die veroorzaakt worden door gebruikelijke micro-organismen op de derde dag na de geboorte.

De langere halfwaardetijd in (premature) neonaten is gunstig voor de

behandeling met antibiotica met een tijdsafhankelijk werkingsmechanisme, omdat hierdoor de tijd dat de concentratie boven de MRC blijft zal toenemen. De langere halfwaardetijd wordt vermoedelijk veroorzaakt doordat bij neonaten de nieren nog niet volledig ontwikkeld zijn. Deze bevinding ondersteunt onze mening dat de eerder beschreven klinische maatstaf voor effect (d.w.z. een tijdsduur van 4 uur tussen de antibioticum toediening en de geboorte) heroverwogen dient te worden. Nadat de hoogste concentratie in het foetale bloed bereikt is, zal de concentratie afnemen. Omdat de halfwaardetijd in de foetus waarschijnlijk korter is dan in de neonaat, zal de hoogste waarde voor T>MRC bereikt worden wanneer het kind geboren wordt kort nadat de hoogste antibioticum concentratie bereikt is.

Toekomstperspectieven

De laatste tijd is het gebruik van antibiotica rondom de bevalling ter preventie van GBS-ziekte toegenomen. Deze toename is deels toe te schrijven aan de verandering van de richtlijnen in de Verenigde Staten van Amerika naar een preventiestrategie die op screening gebaseerd is en aan een toename van de prevalentie van GBS-dragerschap onder zwangere vrouwen. Met het ter beschikking komen van detectie methoden met een hogere sensitiviteit, zullen ook vrouwen met een gering aantal groep B streptokokken geïdentificeerd worden als GBS-draagster. Het is de vraag of ook deze vrouwen een verhoogd risico hebben op een kind met GBS-ziekte. Met het toenemende aantal dat in aanmerking komt voor antibiotische profylaxe tijdens de bevalling, wordt het des te belangrijker dat het geadviseerde doseringsschema adequaat is voor de gehele populatie. Met een suboptimale behandeling kan profylaxe falen en dit zou selectie van resistente bacteriële stammen in de hand kunnen werken.

Uitgaande van het onderzoek dat beschreven wordt in dit proefschrift, is het waarschijnlijk dat de huidige doseringschema's die gebruikt worden voor amoxicilline adequaat zijn bij gezonde zwangere vrouwen, ook wanneer geringe fouten in de toediening worden gemaakt. Maar om zeker te zijn dat deze dosering ook adequaat is voor de foetus dienen simulatie studies uitgevoerd te worden die gebruik maken van het 5-compartimenten model met de farmacokinetische gegevens van de moeder, het kind evenals de gegevens van de inter-individuele variabiliteit. Op deze manier kan een optimaal doseringsschema gekozen worden voor de gehele populatie. Helaas zijn er op dit moment nog geen computerprogramma's beschikbaar die deze simulaties kunnen uitvoeren.

De patiënten die in onze studie geïncludeerd werden, waren op één na allemaal relatief gezond. Bij deze gezonde patiënten konden we aantonen dat een aantal fysiologische factoren invloed heeft op de farmacokinetiek van amoxicilline. Zo heeft de hoeveelheid oedeem invloed op het verdelingsvolume. Zwangerschap

en de bevalling worden soms gecompliceerd door specifieke aandoeningen. Een van deze zwangerschapsgerelateerde aandoeningen in pre-eclampsie, waarbij bekend is dat de hoeveelheid oedeem toeneemt. Hierbij kan de hoeveelheid extracellulair vocht toenemen met 10 liter. Of de huidige doseringsschema's ook adequaat zijn bij patiënten met pre-eclampsie is dus niet zeker. Dit geeft aan dat het belangrijk is om ook farmacokinetisch onderzoek te doen bij patiënten met zwangerschapsgerelateerde aandoeningen.

Voor clindamycine waren slechts weinig data beschikbaar. Wanneer rekening gehouden wordt met de eiwitbinding zou het kunnen zijn dat de concentraties die in de moeder en de foetus bereikt worden niet adequaat zijn om GBS-ziekte te voorkomen. Omdat alleen de fractie van de antibioticumconcentratie die niet aan eiwit gebonden is de placenta kan passeren, kunnen veranderingen in het percentage eiwitbinding invloed hebben op de effectiviteit van de clindamycine. Het is bekend dat de eiwitbinding van clindamycine afhangt van zowel de concentratie alfa1-zure glycoproteine, die verandert tijdens de zwangerschap, als van de clindamycine concentratie zelf. Helaas is er voor het bepalen van de eiwitbinding een relatief grote hoeveelheid bloed nodig. De bloedmonsters afgenomen in het kader van onze studie bleken hiervoor te klein te zijn. Om de effectiviteit van clindamycine voor de preventie van GBS-ziekte aan te tonen, is verder onderzoek nodig. In deze studie dient dan voldoende bloed afgenomen te worden voor het verrichten van bepalingen van de eiwitbinding en tevens dienen patiënten met zwangerschapsgerelateerde aandoeningen geïncludeerd te worden. Met deze gegevens kan dan een farmacokinetisch model ontwikkeld worden dat gebaseerd is op de farmacokinetiek in de moeder en in de foetus, de inter-individuele variabiliteit en de eiwitbinding van clindamycine. Met behulp van dit model en simulatie studies kan dan ook het optimale doseringsschema voor clindamycine bepaald worden.

In de Verenigde Staten van Amerika is Escherichia coli een steeds frequenter voorkomende oorzaak geworden van neonatale infectie, vooral in premature neonaten. Omdat bij deze stammen resistentie tegen amoxicilline vaak voorkomt, zal in de toekomst wellicht de combinatie van amoxicilline met clavulaanzuur meer voorgeschreven gaan worden. We hebben laten zien dat de farmacokinetiek van amoxicilline niet beïnvloed wordt door het tegelijkertijd toedienen van clavulaanzuur, maar voor een goede werking is ook een minimale hoeveelheid clavulaanzuur nodig. Farmacokinetische studies naar de farmacokinetiek van clavulaanzuur zijn daarom ook nodig. Bij het bepalen van de concentraties clavulaanzuur dient rekening gehouden te worden met een aantal problemen. Clavulaanzuur is een instabiele verbinding. Idealiter dienen de concentraties dus bepaald te worden direct na afname van het bloedmonster en een speciale methode dient ontwikkeld te worden om te voorkomen dat het clavulaanzuur tijdens de bepaling afgebroken wordt.

Concluderend kan gezegd worden dat het huidige onderzoek laat zien dat het doseringsschema dat momenteel gebruikt wordt ter preventie van neonatale GBS-ziekte hoogst waarschijnlijk effectief is. Maar om de gebruikte profylaxe te optimaliseren dient meer onderzoek verricht te worden naar de farmacokinetiek van de verschillende antibiotica. Bij dit onderzoek is het belangrijk dat zowel de farmacokinetiek in de moeder als die in het kind, hun onderlinge relatie, de inter-individuele variabiliteit en de eiwitbinding tegelijkertijd in kaart gebracht worden. Daarnaast dienen ook patiënten met zwangerschapsgerelateerde aandoeningen geïncludeerd te worden. Omdat onze studie geen zekerheid kon bieden over de effectiviteit van clindamycine, blijft preventie met een penicilline de voorkeur houden. Wanneer een patiënt aangeeft dat zij allergisch is voor penicilline, kan een allergietest uitgevoerd worden om zeker te zijn dat penicillines gecontraïndiceerd zijn.

Authors and their affiliations

Promotores:

Meindert Danhof

Leiden-Amsterdam Center for Drug Research, Leiden University,
Division of Pharmacology,
P.O. Box 9502,
2300 RA Leiden, the Netherlands.

LAP&P Consultants BV,
Archimedesweg 31,
2333 CM Leiden, the Netherlands.

Eric A.P. Steegers

Erasmus MC, University Medical Centre Rotterdam,
Department of Obstetrics and Gynecology, Division of Obstetrics and Prenatal Medicine.
's-Gravendijkwal 320,
3015 CE Rotterdam, the Netherlands.

Co-promotores:

P. Joep Dörr

Medical Center Haaglanden (MCH),
Department of Obstetrics and Gynecology,
Lijnbaan 32,
2512 VA The Hague, the Netherlands.

Johan W. Mouton

Canisius Wilhelmina Hospital,
Department of Clinical Microbiology and Infectious Diseases,
Weg door Jonkerbos 100,
6532 SZ Nijmegen, the Netherlands.

Others:

John N. van den Anker

Children's National Medical Center,
Division of Pediatric Clinical Pharmacology,
111 Michigan Avenue, N.W.,
Washington, DC 20010, USA.

Erasmus MC-Sophia, Sophia Children's Hospital,
's-Gravendijkwal 320,
3015 CE Rotterdam, the Netherlands.

Ymka Bult
Wilhelmina Hospital Assen,
Department of Pediatrics,
Europaweg-Zuid 1,
9401 RK Assen, the Netherlands.

Wil H.F. Goessens
Erasmus MC, University Medical Centre Rotterdam, Department of Medical Microbiology and infectious Diseases,
's-Gravendijkwal 320,
3015 CE Rotterdam, the Netherlands.

Joost DeJongh
LAP&P Consultants BV,
Archimedesweg 31,
2333 CM Leiden, the Netherlands.

Leiden-Amsterdam Center for Drug Research, Leiden University,
Division of Pharmacology,
P.O. Box 9502,
2300 RA Leiden, the Netherlands.

Lia (C) Liefaard
LAP&P Consultants BV,
Archimedesweg 31,
2333 CM Leiden, the Netherlands.

Paul M. Oostvogel
Medical Center Haaglanden (MCH),
Department of Clinical Microbiology,
Lijnbaan 32,
2512 VA the Hague, the Netherlands.

Rob A. Voskuyl
Leiden-Amsterdam Center for Drug Research,
Division of Pharmacology, Leiden University,
P.O. Box 9502,
2300 RA Leiden, the Netherlands

SEIN- Epilepsy Institutes of the Netherlands Foundation
Achterweg 5
2103 SW, Heemstede, the Netherlands

Nawoord

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Vanuit Den Haag was ik mij er wel degelijk van bewust dat veel werk elders in het land gebeurde. Henny van Daal, zeer veel dank voor het verrichten van de HPLC bepalingen in Nijmegen. Nieko Punt, veel dank voor alle inzet voor het tot stand laten komen van het computerprogramma voor het uitvoeren van de simulaties in Maastricht.

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Marissa Vrolijk-de Mos en Nanouk Wiemer, alledrie deden we onderzoek. Weliswaar in een ander vakgebied, maar we begrepen elkaar altijd goed. Jullie zijn steeds een grote steun voor mij geweest. Marissa, veel dingen hebben we samen meegemaakt tijdens de middelbare schooltijd, studie geneeskunde, promotieonderzoek en nu nog tijdens de opleiding tot medisch specialist. Nanouk, jij had mijn telefoonnummer gekregen voor informatie over een stage in Australië. Sindsdien zijn we goede vriendinnen. Ik heb naast jou gestaan tijdens jouw promotie en jij zult naast mij staan.
Ik ben blij dat ik zulke goede vriendinnen heb.

Mijn ouders ben ik dankbaar voor de voortdurende steun die ze me hebben gegeven. Bedankt voor de kansen die ik van jullie heb gekregen.

Curriculum Vitae

Anouk Muller werd geboren op 17 mei 1977 te Den Haag. Na het behalen van het diploma gymnasium in 1995 aan het Gymnasium Haganum te Den Haag begon zij met de studie geneeskunde. De studie werd aangevangen in 1996 aan het Rijks Universitair Centrum Antwerpen in België en werd vanaf 1997 aan de Universiteit Leiden voortgezet. Tijdens haar studie was zij betrokken bij het groep B streptokokken onderzoek in het Medisch Centrum Haaglanden in Den Haag (Dr. P.J. Dörr, Dr. P.M. Oostvogel en anderen). Zij deed daarna enkele maanden extra wetenschappelijk onderzoek naar de rol van IL-6 bij de folliculaire ontwikkeling van de ovaria bij muizen aan de University of Adelaide, Australië (Prof. dr. F.M. Helmerhorst en Dr. S.A. Robertson). Tijdens de co-schappen (vanaf 2002) deed zij een literatuuronderzoek naar de farmacokinetiek van antibiotica tijdens de zwangerschap. Dit resulteerde in de onderzoeksvraag voor haar promotieonderzoek. Na haar artsexamen in 2004 werkte zij als ANIOS op de afdeling verloskunde en gynaecologie van het Medisch Centrum Haaglanden. Van november 2004 tot en met september 2007 werkte zij als assistent in opleiding (AIO) in het Medisch Centrum Haaglanden onder begeleiding van promotores Prof. Dr. M. Danhof (Leiden) en Prof. dr. E.A.P. Steegers (Rotterdam) en van co-promotores Dr. P.J. Dörr (Den Haag) en Dr. J.W. Mouton (Nijmegen) aan het onderzoek beschreven in dit proefschrift. Sinds oktober 2007 is zij werkzaam als arts-assistent in opleiding tot arts-microbioloog in het Maasstad Ziekenhuis (opleider: Dr. W.D.H. Hendriks) en het Erasmus Medisch Centrum (opleider: Prof. dr. H.A.Verbrugh) te Rotterdam.

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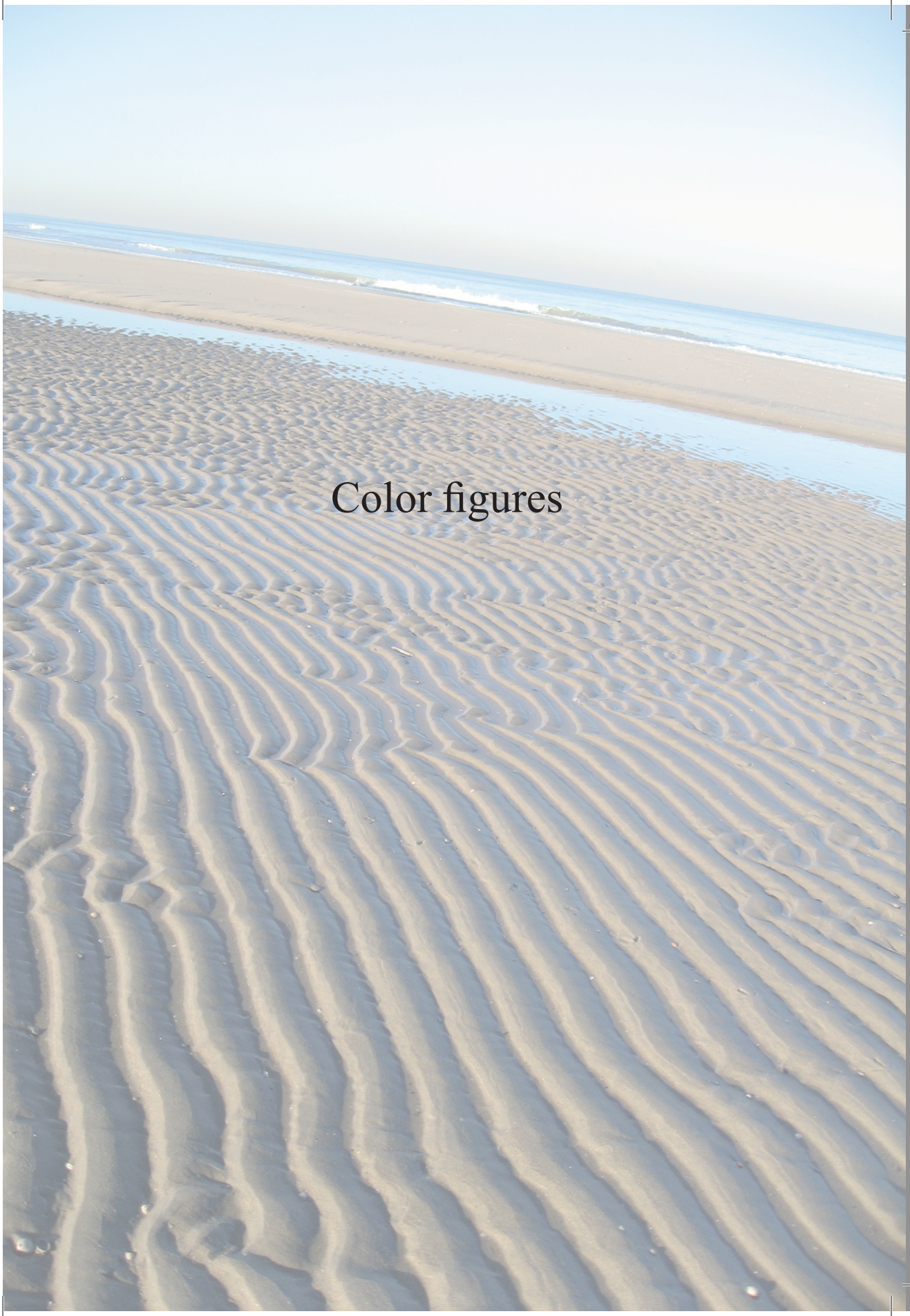
Muller AE, Oostvogel PM, DeJongh J, Mouton JW, Steegers EA, Dörr PJ, Danhof M, Voskuyl RA. Pharmacokinetics of amoxicillin in maternal, umbilical cord and neonatal serum.
Submitted



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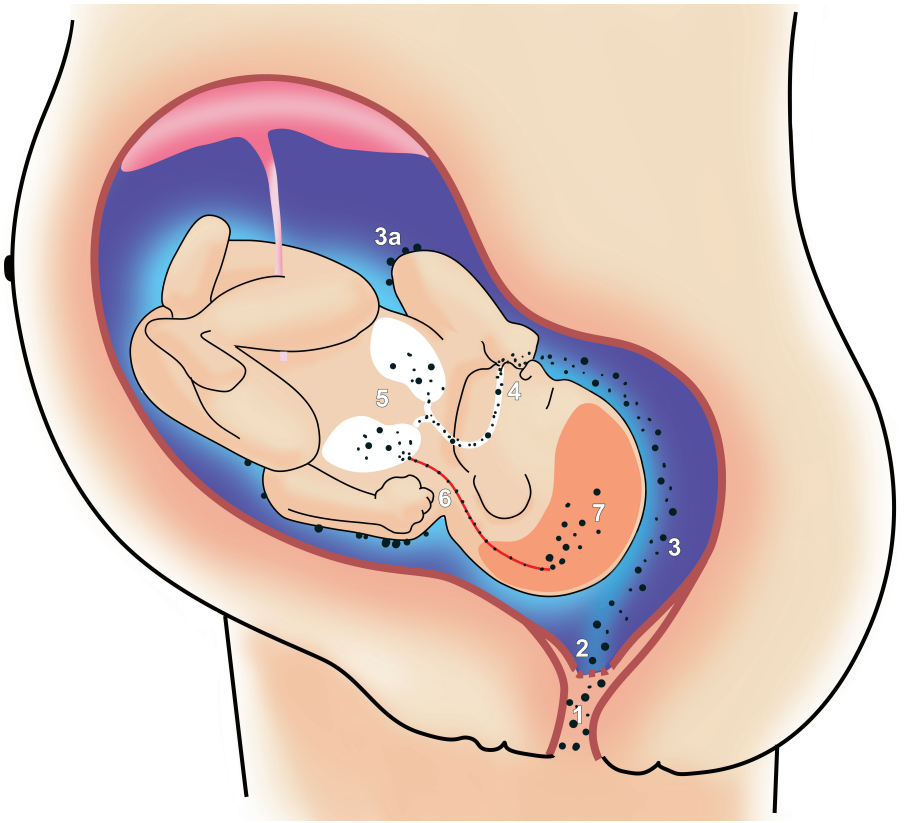
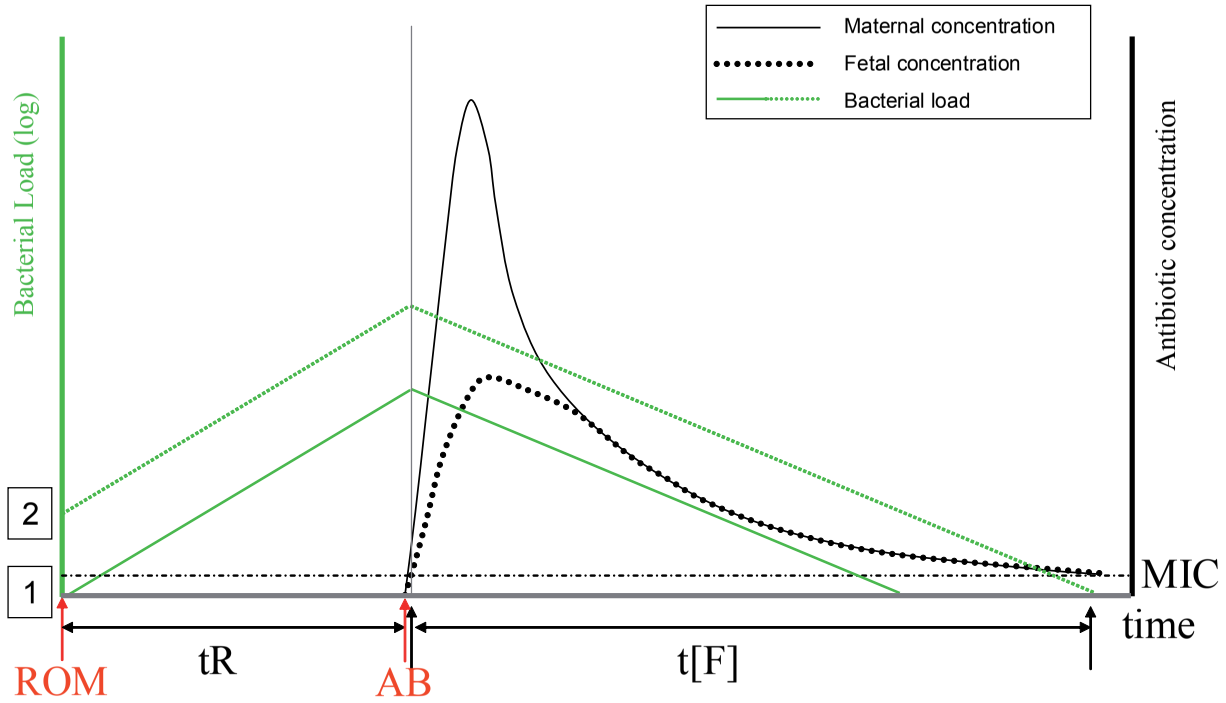


Designed by Vincent Khouw (VMK designs)

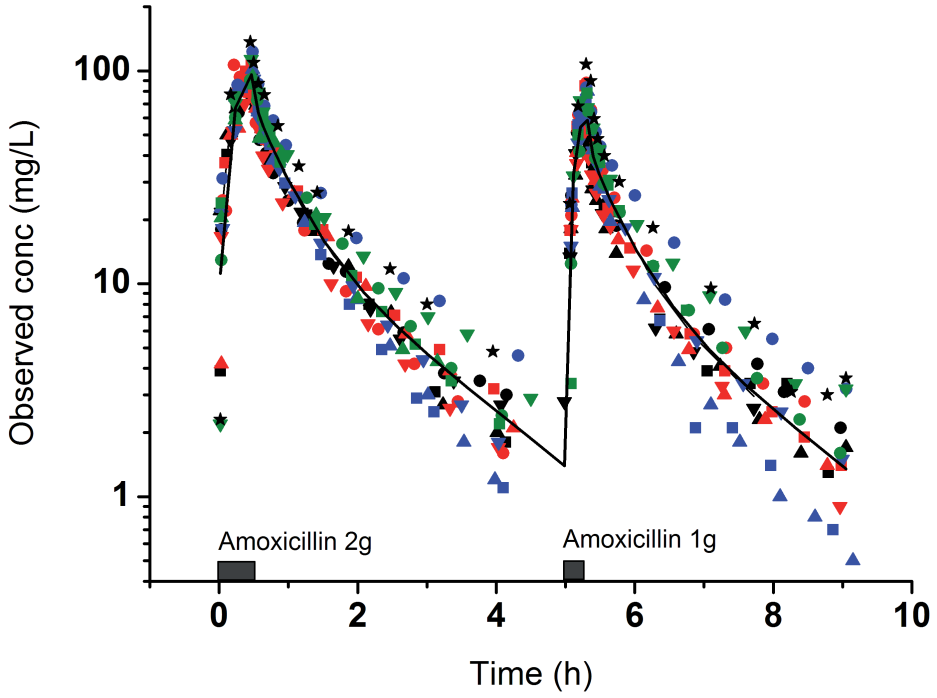
***Chapter 2, Figure 1**: Hypothesized pathogenesis of GBS-EOD.*

1 Colonization in the rectovaginal compartment; 2 Rupture of the membranes; 3 GBS enters the amniotic fluid; 3a GBS colonization of skin and mucocutaneous areas; 4 Aspiration of infected amniotic fluid; 5 Infected amniotic fluid causes pneumonia (if the bacterial load is high enough); 6 Entry of GBS in the bloodstream (sepsis or bacteremia); 7 Entry in cerebrospinal fluid after hematogenous spread (meningitis).



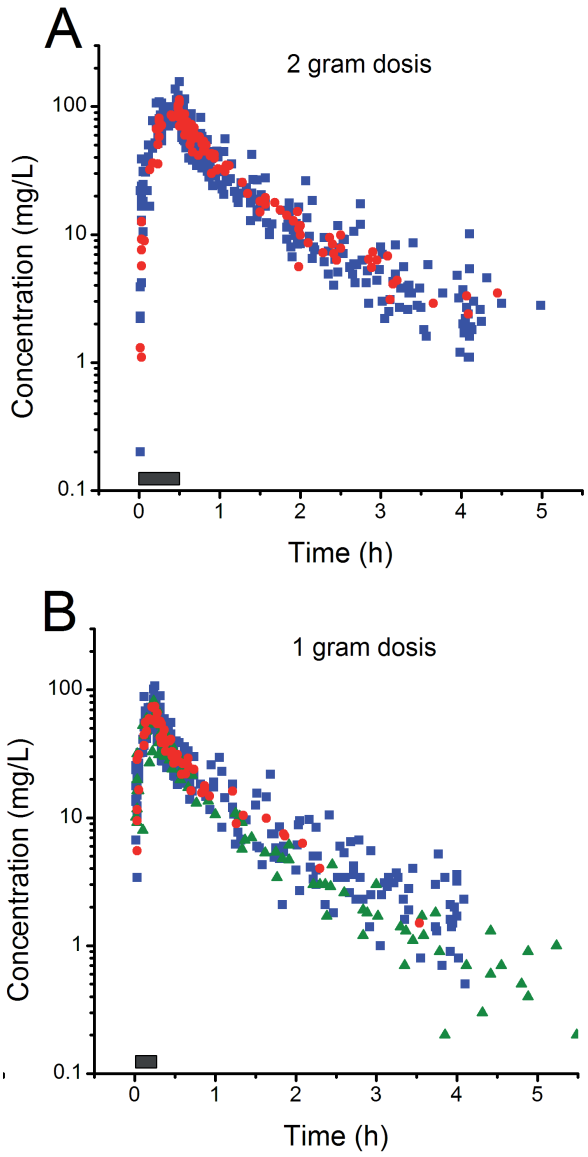
Chapter 2, Figure 2: *The effect of antibiotic prophylaxis on the bacterial load of GBS.*

ROM= Rupture of membranes, tR= time between ROM and start of antibiotics, AB=start of administration of antibiotic, MIC=minimum inhibitory concentration, t[F]= time the fetal concentration exceeds the MIC; 1 changes in bacterial load. 2 enhanced bacterial load in patients in maternal fever or prolonged ROM

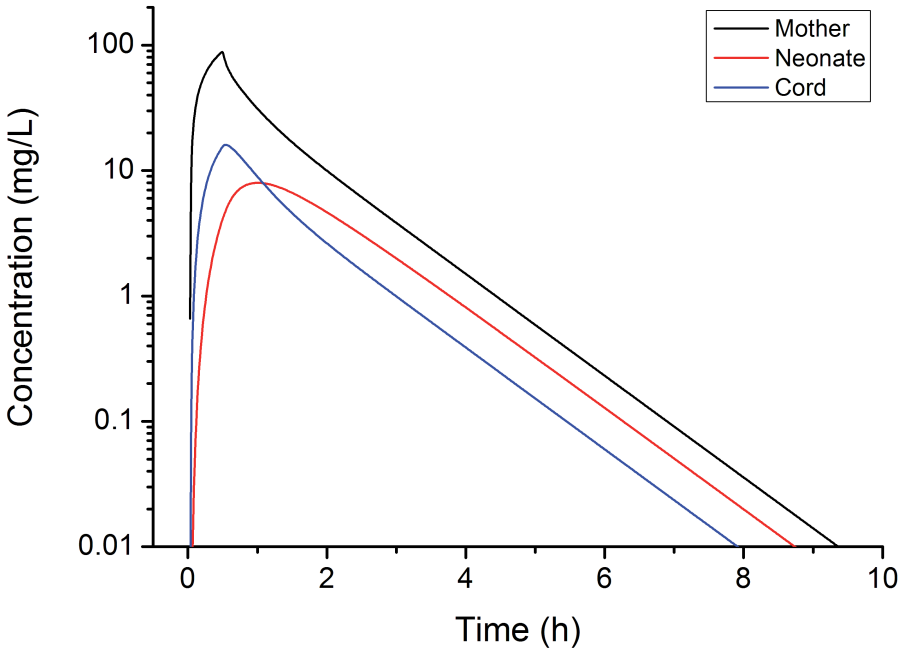


***Chapter 4, Figure 1**: Observed concentration-time profiles in patients with PPROM.*

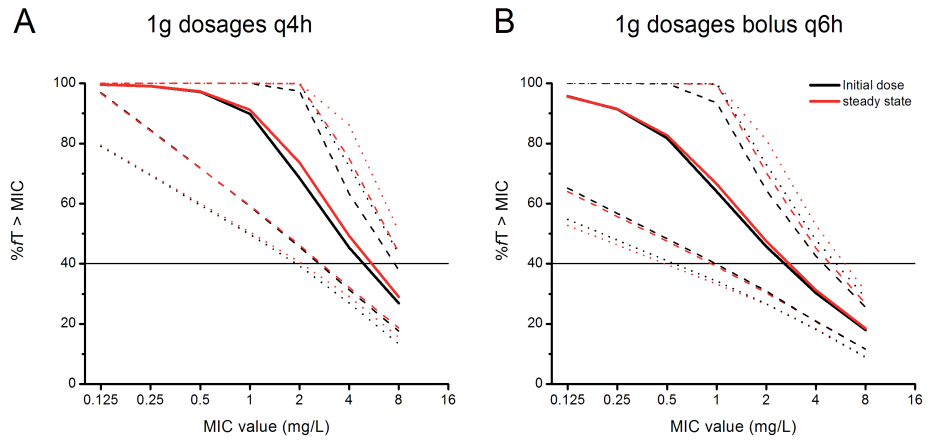
The superimposed bold line shows the predicted profile obtained with the final model. The blocks indicate the time at which the infusions of the amoxicillin was started and stopped. Because there was variation in the start-time of the second infusion due to the clinical situation, in this graph the data were adapted assuming that the second infusions started at t=5.05h for all patients.



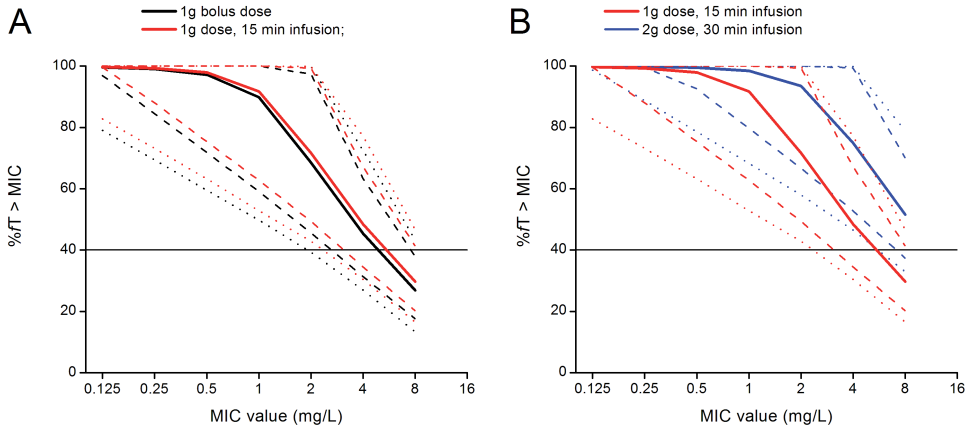
Chapter 5, Figure 3: *Figure 3a shows the observed amoxicillin concentrations after a 2 gram dose and figure 3b after a 1 gram dose. Time of infusion is indicated by black bars. The blue squares represent all data points of the patients before the onset of labor; the red dots data points of patients during labor and data points of patients in the postpartum period are indicated by the green triangles. Because there was variation in the start-time of the second infusion, in figure 3b the data were adapted assuming that the 1 gram infusions started at t=0 for all patients.*



Chapter 7, Figure 4: *Simulated concentration-time profiles for the mother, umbilical cord and neonate.*
Concentration-time profile of amoxicillin in maternal, umbilical cord and neonatal serum simulated after a single dose of 2 gram amoxicillin infused over 30 minutes. The simulations were performed with PK parameter estimates based on the final 5-compartment model and carried out for 12 hours after a single antibiotic dose.



Chapter 8, Figure 2: *Percent of time the unbound fraction of amoxicillin remained above the MIC (%fT>MIC) as a function of the MIC for two dosing intervals, 4 hours (figure 2a) and 6 hours (figure 2b), in pregnant women with PPROM after the inital dose (black lines) and in steady state situation (red lines).*



Chapter 8, Figure 3: *Percent of time the unbound fraction of amoxicillin remained above the MIC (%fT>MIC) as a function of the MIC for three different initial doses for a 4 hours dosing interval in pregnant women with PPROM. In figure 3A the %fT>MIC for a dose of 1 gram administered as bolus (black lines) and the 1 gram dose administered over 15 minutes (red lines) are shown. In figure 3B the %fT>MIC for the 1 gram dose administered over 15 minutes (red lines) and the 2 gram dose administered over 30 minutes (blue lines) are shown. The solid lines are the values for the average pregnant women; the interrupted lines represent the 95% confidence intervals and the dotted lines the 99% confidence intervals.*